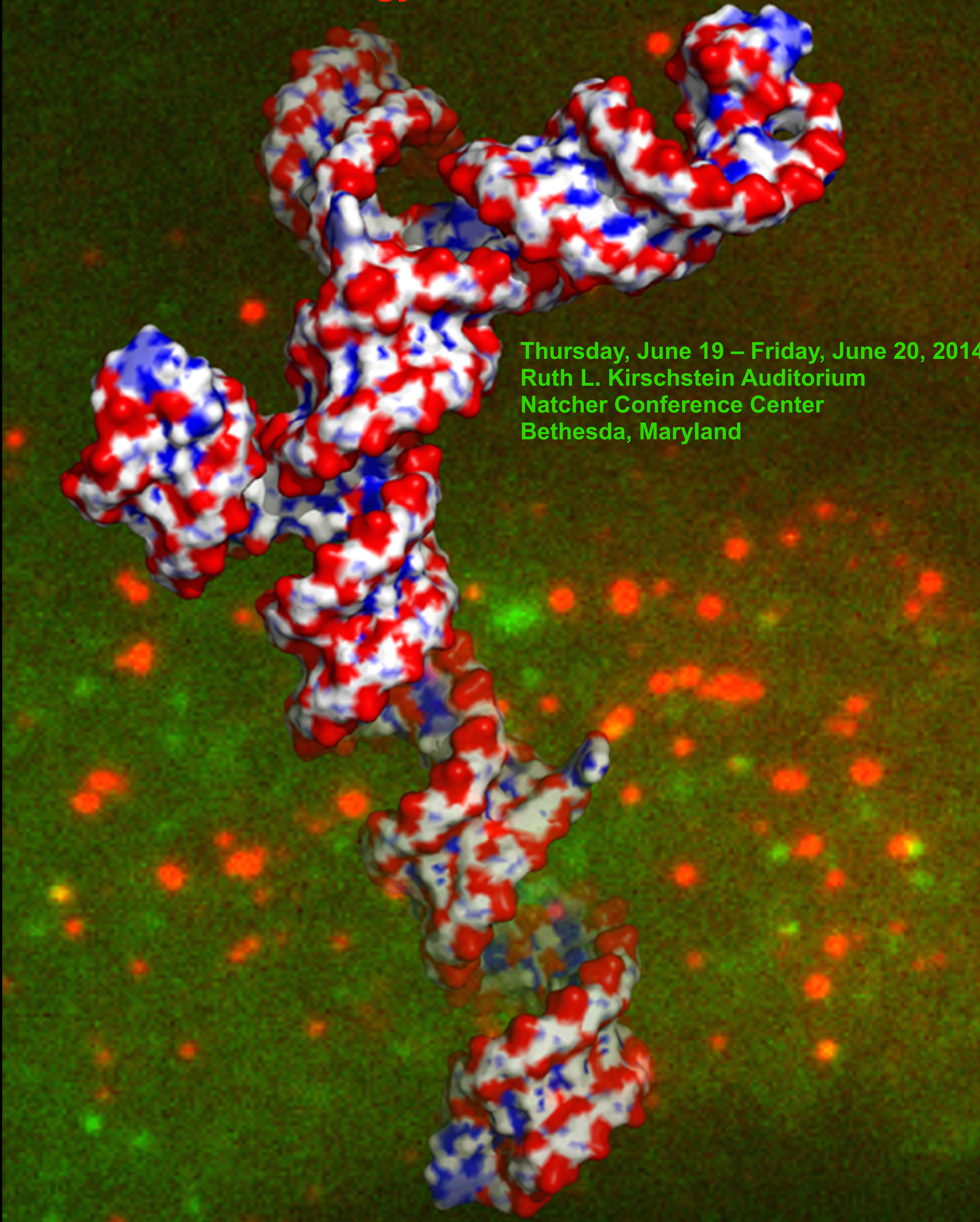
Structural Biology Related to HIV/AIDS - 2014



Thursday, June 19 – Friday, June 20, 2014
Ruth L. Kirschstein Auditorium
Natcher Conference Center
Bethesda, Maryland

NIH National Institute of General Medical Sciences

NIH National Institute of Allergy and Infectious Diseases

About the Cover:

Foreground: NMR structure of the portion of the HIV-1 genome that directs genome packaging (P and O atoms colored red; C and H atoms colored white; N atoms colored blue).

Background: Image of a HeLa cell showing HIV-Gag (red) and HIV RNA (green) at sites of assembly on the plasma membrane using TIR illumination. Green densities that are initially detected gradually turn yellow, then red, as large numbers of Gag molecules associate with two copies of RNA.

Raw Images provided by the CRNA-associated laboratories of M.F. Summers and S. Simon.

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Structural Biology Related to HIV/AIDS – 2014

DAY 1

8:00 – 8:05 **Michael Sakalian** (National Institute of General Medical Sciences)
Welcome/Opening Remarks

Session I: Reports from Specialized Centers (P50); Session Chair: Reuben Harris

8:05 – 9:15 The Center for HIV RNA Studies (CRNA)
Alice Telesnitsky (University of Michigan)
Introduction to the CRNA

Blanton Tolbert (Case Western Reserve University)
RNA Structures and Protein Interactions Involved in HIV Splicing

Paul Bieniasz (Aaron Diamond AIDS Research Center)
Global Identification of RNA Sequences Bound by HIV-1 Gag During Virion Genesis

Michael Summers (University of Maryland Baltimore County)
NMR Structure of the HIV-1 Core Encapsidation Signal

9:15 – 10:25 The Pittsburgh Center for HIV Protein Interactions (PCHPI)
Angela Gronenborn (University of Pittsburgh)
Introduction to the PCHPI

Tatyana Polenova (University of Delaware)
Magic Angle Spinning NMR of HIV-1 Protein Assemblies: Insights into Structure and Dynamics

Zandrea Ambrose (University of Pittsburgh School of Medicine)
Confocal and Super Resolution Imaging of HIV-1 Uncoating and Nuclear Entry

Yong Xiong (Yale University)
Molecular Insight into HIV Restriction by Myxovirus Resistance Protein 2 (MxB)

10:25 – 11:00 BREAK

Session II: Reports from Specialized Centers (P50) continued; Session Chair: Celia Schiffer

11:00 – 12:10 The HIV Interaction and Viral Evolution Center (HIVE)
Arthur Olson (The Scripps Research Institute)
Introduction to the Center

Mamuka Kvaratskhelia (Ohio State University)
A New Class of Multimerization Selective Inhibitors of HIV-1 Integrase

Bruce Torbett (The Scripps Research Institute)
Deep Sequencing of Drug Resistance HIV Reveals Correlated Mutation

Steven Tuske (Rutgers University)
Dynamics of HIV-1 Reverse Transcriptase Interactions with Nucleic Acid

Stefan Sarafianos (University of Missouri, Columbia)
Crystal Structure of the Native Hexameric Full-Length HIV-1 Capsid

12:10 – 1:00 LUNCH

1:00 – 3:00 POSTER SESSION

Session III: Reports from Specialized Centers (P50) continued; Session Chair: Irwin Chaiken

3:00 – 4:10 The Center for HIV Accessory and Regulatory Complexes (HARC)
Alan Frankel (University of California, San Francisco)
Introduction to the Center

Ursula Schulze-Gahmen (University of California, Berkeley)
AFF4 Binding to the Tat-P-TEFb Stimulates TAR Recognition at the HIV Promoter

Alan Frankel (University of California, San Francisco)
Architecture of the HIV RNA Export Complex

Yfan Cheng (University of California, San Francisco)
EM Structures of Rev Complexes and Beyond

4:10 – 5:20 The Center for the Structural Biology of Cellular Host Elements in Egress, Trafficking, and Assembly of HIV (CHEETAH)
Wes Sundquist (University of Utah)
Introduction to the Center

Pamela Bjorkman (California Institute of Technology)
Electron Tomography of HIV-1 Infection in Gut-Associated Lymphoid Tissue

Adam Frost (University of Utah)
Structure of an ESCRT-III Membrane-Remodeling Complex

Dmitri Ivanov (University of Texas Health Science Center at San Antonio)
Activation of the Ubc13-Ub Adduct by TRIM5alpha Requires Dimerization of the RING Domain

5:20 ADJOURN FOR DAY

DAY TWO

8:00 – 8:05 **Jon Lorsch** (Director, NIGMS)
Welcome/Day 2

Session IV: Trafficking and Assembly, Session Chair: Wes Sundquist

8:05 – 8:35 **Jennifer Lippincott-Schwartz** (NIH/NICHD)
Cell Biological Mechanisms Underlying HIV-1 Viral Particle Assembly and Release

8:35 – 9:05 **Mark Marsh** (MRC/University College, London)
The IPMC - a Macrophage Compartment Exploited by HIV for Virus Assembly

Session V: Membrane Proteins, Session Chair: Arthur Olson

9:05 – 9:35 **Michael Kent** (Sandia National Laboratories)
Conformational Transition of Membrane-Associated Terminally Acylated HIV-1 Nef and Its Interactions with Host Proteins

9:35 – 10:05 **Ian Wilson** (The Scripps Research Institute)
HIV-1 Env Trimer and Interaction with Broadly Neutralizing Antibodies

10:05 – 10:30 BREAK

Session V: Membrane Proteins continued, Session Chair: Arthur Olson

10:30 – 11:15 P01: Structure-Based Antagonism of HIV-1 Envelope Function in Cell Entry
Irwin Chaiken (Drexel University)
Introduction: HIV-1 Antagonism and Inactivation Through Env gp120 Targeting

Joseph Sodroski (Dana Farber Cancer Institute)
Structure, Function and Inhibition of the HIV-1 Envelope Glycoproteins

Session VI: APOBEC, Session Chair: Alice Telesnitsky

11:15 – 12:00 P01: Critical Interactions of APOBEC3s: Molecular Approaches to Novel HIV Therapies
Reuben Harris (University of Minnesota)
HIV-1 Restriction in Primary T Cells by Stable APOBEC3H Haplotypes

Hiroshi Matsuo (University of Minnesota)
NMR Structure of the HIV-1 Vif Binding Domain of APOBEC3G

Celia Schiffer (University of Massachusetts Medical School)
Structural Studies of APOBEC3s

12:00 – 1:00 LUNCH
(SAB Lunch in 2As.10; Please bring your lunch to the second floor.)

1:00 – 3:00 POSTER SESSION
(1:00 – 3:00) NIGMS Centers Scientific Review Board; Room 2As.10; second floor)

Session VII: Special Presentation on Cure Targets, Session Chair: Elizabeth Stansell, NIAID

3:00 – 3:30 **Jonathan Karn** (Case Western Reserve University)
Molecular Targets Controlling HIV Latency and Their Exploitation in Cure Strategies

Session VIII: Transcription and RNA:, Session Chair: Alan Frankel

3:30 – 4:00 **Yun-Xing Wang** (NIH/NCI/CCR)
The Topological Structure of the HIV-1 Rev Response Element RNA

4:00 – 4:30 **Tahir Tahirov** (University of Nebraska Medical Center)
2.9 A Resolution Crystal Structure of HIV-1 Tat Complexed with Human P-TEFb and AFF4

Session IV: Protease, Session Chair: Angela Gronenborn

4:30 – 5:00 The Interdependency of Drug Resistance Evolution and Drug Design: HIV-1 Protease
Nese Kurt-Yilmaz (University of Massachusetts Medical School)
Project Overview

Ronald Swanstrom (University of North Carolina Chapel Hill School of Medicine)
Selection for PI Drug Resistance in Cell Culture: Better Than You Think

Woody Sherman (Schrodinger, Inc.)
The Role of Water in Molecular Recognition and Selectivity

5:00 ADJOURN MEETING

Mark Your Calendars!

Structural Biology Related to HIV/AIDS – 2015

Thursday June 18 – Friday June 19, 2015

Natcher Conference Center, Bethesda, Maryland

About the Poster Sessions

Thursday	**1:00 – 3:00**	**T-numbered posters**
Friday	**1:00 – 3:00**	**F-numbered posters**

Please remove your poster from the board at the end of the assigned day. This year there are too many for posters to be left up for the whole meeting.

Thursday Posters

Tat, TAR, and Transcription

T1. Reactivation of Latent HIV-1 Provirus via Targeting Protein Phosphatase-1

Sergei Nekhai[1], Mudit Tyagi[2], Sergei Iordansky[3], Kahli Smith[1], Namita Kumari[1], Marina Jerebtsova[4], Tatyana Ammosova[1], Dmytro Kovalskyy[5], Fatah Kashanchi[3], and Michael Petukhov[6]

[1]Center for Sickle Cell Disease, Departments of Medicine, Howard University, Washington DC 20059; [2]The George Washington University, 2300 Eye Street, N.W. Washington, DC 20037; [3]National Center for Biodefense and Infectious Diseases, George Mason University, 10900 University Blvd., Manassas, VA 20110; [4]Center for Genetic Medicine, Children's National Medical Center, 111 Michigan Ave, N.W., Washington DC 20010; [5]ChemBio Center, National Taras Shevchenko University, Kiev, Ukraine; and [6]Division of Molecular and Radiation Biophysics, Petersburg Nuclear Physics Institute, Gatchina, Russia

T2. Purification of 7SK RNP Containing the HIV Tat Co-Factors Cdk9 And CyclinT1

Hongbing Liu and Andrew P. Rice

Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX

T3. Characterization of the HIV-1 Tat – P-TEFb Interaction Using Global Substrate Profiling

Nicole Olson, Anthony O'Donoghue, Giselle Knudsen, Ursula Schulze-Gahmen, and Charles Craik

University of California, San Francisco

T4. Uncovering the Underlying Mechanisms of HIV Transactivation Induced by Post-Translational Modifications in the TAT:AFF4:P-TEFb Semi-Super Elongation Complex

John Bruning[1], Ursula Schulze-Gahmen[2], and Matt Jacobson[1,3]

[1]Department of Pharmaceutical Sciences and Pharmacogenomics, University of California San Francisco, San Francisco, CA, 94158; [2]Department of Molecular and Cell Biology, University of California Berkeley, Berkeley, CA, 94720; [3]Department of Biophysics, University of California San Francisco, San Francisco, CA, 94158.

T5. Picomolar Ligand for TAR RNA Inhibits HIV Replication in Primary Lymphocytes with Activity Comparable to Current Antivirals

Alisha Jones, Matthew D. Shortridge, Amy Davidson, Jordan Yip, Matt Lalonde, Eric Arts, Jon Karn, John Robinson, and Gabriele Varani

Department of Chemistry, University of Washington

T6. Dynamic-Ensemble Based Targeting of HIV-1 RNA

Janghyun Lee[1], Shan Yang[1], Bharathwaj Sathyamoorthy[1], Steven J. Soll[2], Paul D. Bieniasz[2] and Hashim M. Al-Hashimi[1]

[1]Department of Biochemistry, Duke University Medical Center, Durham, NC 27710, USA; [2]Aaron Diamond AIDS Research Center, The Rockefeller University, New York, NY 10016, USA

T7. RNA-Binding Small Molecules Using Small Molecule Microarrays

Joanna Sztuba-Solinska,[†] *Shilpa R. Shenoy,*[‡] *Peter Gareiss,*[§] *Lauren H. Krumpe,*[‡] *Stuart Le Grice,*[†] *Barry O'Keefe,*[‡] *and John S. Schneekloth, Jr.*[#]
[†]*HIV Drug Resistance Program, National Cancer Institute, Frederick, MD, USA;* [‡]*Molecular Targets Laboratory, National Cancer Institute, Frederick, MD, USA;* [§]*Center For Molecular Discovery, Yale University, New Haven CT, USA;* [#]*Chemical Biology Laboratory, National Cancer Institute, Frederick, MD, USA*

T8. A Biophysical Characterization Study of a Potent Small Molecule HIV Inhibitor With Its Target: Didehydro-Cortistatin A/Tat HIV

Sonia Mediouni[1], Victoria D'Souza[2], Douglas Kojetin[3], Susana Valente[1]
[1]*Department of Infectology, the Scripps Research Institute, Florida, USA;* [2]*Department of Molecular and Cellular Biology, Harvard University, Massachusetts, USA;* [3]*Department of Molecular Therapeutics, the Scripps Research Institute, Florida, USA.*

RNA Splicing

T9. Finally: Efficient Quantification of HIV-1 Splicing

Ann Emery[1], Ronald Swanstrom[2]
[1]*Genetics and Molecular Biology Curriculum,* [2]*Department of Biochemistry and Biophysics, University of North Carolina Chapel Hill*

T10. Undiscovered Cis-Acting RNA Elements in the HIV-1 Genome

Steven J Soll[1,2,3], Daniel Blanco-Melo[1,3], Paul D Bieniasz[1,2,3]
[1]*Aaron Diamond AIDS Research Center, The Rockefeller University, New York, NY, 10016;* [2]*Howard Hughes Medical Institute, The Rockefeller University, New York, NY, 10016;* [3]*The Rockefeller University, Laboratory of Retrovirology, New York, NY, 10016*

T11. Structural Characterization of the Intronic Splicing Silencer (ISS) of HIV-1 Using NMR

Niyati Jain, Christopher E. Morgan and Blanton S. Tolbert
Case Western Reserve University, jxn172@case.edu

T12. Elucidation of hnRNP A1 Binding to ssA7 of HIV-1

Jeffrey D. Levengood[1], Jennifer Meagher[2], Carrie Rollins[1], Jeanne Stuckey[2], and Blanton Tolbert[1]
[1]*Department of Chemistry, Case Western Reserve University, Cleveland, OH;* [2]*Life Sciences Institute, University of Michigan, Ann Arbor, MI*

Rev, RRE, and DDX1

T13. The HIV-1 Rev Response Element Exists in Two Alternative Secondary Structures Which Promote Different Replication Activities

Chringma Sherpa[1], Jason Rausch[2], Stuart Le-Grice[2], Marie-Louise Hammarskjold[1], and David Rekosh[1]
[1]*Myles H. Thaler Center for AIDS Center, Microbiology Dept., University of Virginia, Charlottesville, VA;* [2]*National Cancer Institute, NIH, Frederick, MD*

T14. Structural Studies of the HIV-1 and HIV-2 Rev Response Elements

Jason W. Rausch[1], Sabrina Lusvarghi[1], Joanna Sztuba-Solinska[1], Chringma Sherpa[2], Marie-Louise Hammarskjold[2], David Rekosh[2] and Stuart F.J. Le Grice[1]

[1]HIV Drug Resistance Program, Reverse Transcriptase Biochemistry Section, Frederick National Laboratory for Cancer Research, Frederick, MD [2]Myles H. Thaler Center for AIDS and Human Retrovirus Research and Department of Microbiology, University of Virginia, Charlottesville, VA

T15. Structural Biology of an HIV-1 Rev-RRE Complex

Bhargavi Jayaraman, David C. Crosby, Christina Homer, Isabel Ribeiro, David Mavor and Alan D. Frankel

HIV Accessory and Regulatory Complex Center (HARC), Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94158

T16. Assembly of Rev and Cofactor DDX1 on the Rev Response Element

Rajan Lamichhane, Ingemar Pedron and David Millar

Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, CA 92037 USA

T17. Roles of DDX1 in Intracellular Trafficking of Unspliced HIV-1 mRNA

Hui-Yi Chu[1], John Hammond[2], Li-Chun Tu[3], Souad Naji, James R. Williamson[2], David Grunwald[3], and Larry Gerace[1]

[1]Depts. of Cell and Molecular Biology, The Scripps Research Institute, La Jolla, CA; [2]Depts. of Molecular Biology and Depts. of Chemistry, The Scripps Research Institute, La Jolla, CA; [3]RNA Therapeutics Institute, Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA

T18. A Mechanistic Role For Human DEAD-Box Helicase 1 (DDX1) in HIV-1 Rev-Dependent Export

John A. Hammond, Li Zhou, David P. Millar, Jamie R. Williamson

The Scripps Research Institute Department of Molecular Biology, 10550 North Torrey Pines Road, La Jolla, California, 92037

T19. Living Cell Real-Time Single Molecule Imaging of HIV-1 RNA Modulation by DDX1

Li-Chun Tu[1], Aviva Joseph[1], Hui-Yi Chu[2], Larry Gerace[2] and David Grunwald[1]

[1]RNA Therapeutics Institute, University of Massachusetts Medical School, Worcester, MA; [2]Department of Cell and Molecular Biology, The Scripps Research Institute, La Jolla, CA

RNA Structure, Function, and Packaging

T20. The Role of HIV-1 Genomic RNA Secondary Structure on Frameshifting *In Vivo.*

Pablo Garcia-Miranda, Jordan T. Becker, Alexander Blume, Nathan M. Sherer and Samuel E. Butcher.

Department of Biochemistry, University of Wisconsin, Madison, WI 53706-1544, USA

T21. Chemo-Enzymatic Labeling Methods to Decode Encoded RNA Messages

Owen Becette[1], Andrew P Longhini[1], Regan M LeBlanc[1], Luigi J Alvarado[1], T. Kwaku Dayie[1,3]
[1]Center for Biomolecular Structure and Organization, Department of Chemistry and Biochemistry, University of Maryland, College Park, MD; [2] Center for HIV RNA Research; University of Michigan Medical School Department of Microbiology and Immunology, Ann Arbor MI 48109-5620

T22. NMR Spectroscopy of Large RNAs Using *In Vivo* Labeled RNAs

Rachel E. Brown[1], My T. Le[2], Andrew P. Longhini[1], and T. Kwaku Dayie[1,3]
[1]Center for Biomolecular Structure and Organization, Department of Chemistry and Biochemistry, University of Maryland, College Park, MD 20742; [2]Department of Molecular and Cell Biology, University of Maryland, College Park, MD 20742; [3] Center for HIV RNA Research, University of Michigan Medical School Department of Microbiology and Immunology Ann Arbor MI 48109-5620

T23. Recent Advances in NMRViewJ for Analysis of RNA

Bayard Fetler[2,] Gaurav Luthria[2], Chris McGee[2], Michael Norris[2], Michael F. Summers[1], Bruce A. Johnson[2]
[1]Howard Hughes Medical Institute, Department of Chemistry and Biochemistry, University of Maryland, Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250; [2]One Moon Scientific, Inc, 839 Grant Ave., Westfield, NJ 07090

T24. RNA Secondary Structure of HIV-1 Gag Using Phylodynamics and Covariation Analysis

Gary B. Fogel[1], Brittany Rife[2], Susanna L. Lamers[3], David J. Nolan[2], Marco Salemi[2]
[1]Natural Selection, Inc., CA, USA; [2]University of Florida, FL, USA; [3]Bioinfoexperts, LLC, LA, USA

T25. Electron Cryo-Microscopy of Retroviral RNA

Irobalieva R[1,2], Keane S[3], Girma D[3], Liu Y[3], Ludtke SJ[1,2], Schmid MF[1,2], Summers MF[3], and Chiu W[1,2]
[1]Graduate Program in Structural and Computational Biology and Molecular Biophysics, Baylor College of Medicine, Houston, TX; [2]National Center for Macromolecular Imaging, Verna and Marrs McLean; Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX; [3]Howard Hughes Medical Institute, Department of Chemistry and Biochemistry, University of Maryland, Baltimore County, Baltimore MD

T26. Selective Pressure at the Level of Structured RNA for HIV-1 p24: The Need for Quantitative Selection Measures

Rife BD,[1] Fogel GB,[2] Lamers SL,[3] Norstrom MM,[4] Nolan DJ,[1] Salemi M[1]
[1]University of Florida, FL, USA; [2]Natural Selection, Inc., CA, USA; [3]BioInfoExperts, LLC, LA, USA; [4]Karolinska Institutet, Stockholm, Sweden

T27. Characterization of the Monomeric Conformation of the HIV-1 RNA 5'-Untranslated Region

Sarah Monti, Amar Kaneria, Verna Van, Nicholas Bolden, and Michael Summers
HHMI, University of Maryland, Baltimore County, USA

T28. Structure of the HIV-1 Core Encapsidation Signal.

Sarah C. Keane, Xiao Heng, Kun Lu, Venkateswaran Ramakrishnan, Shawn Barton, Gregory Carter, Azra Hosic, Alyssa Forwick, Sayo McCowin, Seung H. Choi, Justin Santos, Jan Marchant, Siarhei Kharytonchyk, Alice Telesnitsky and Michael F. Summers.
Howard Hughes Medical Institute and Department of Chemistry and Biochemistry, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250, USA

T29. Distinctions in HIV-1 RNA Structure and Host Components Between the Translation RNP and the Packaging RNP

Ioana Boeras and Kathleen Boris-Lawrie
HIV Center for RNA Studies, Department of Veterinary Biosciences, Center for Retrovirus Research, Center for RNA Biology, The Ohio State University, Columbus Ohio, USA

T30. Global Identification of the RNA Targets of HIV-1 Gag During Virion Genesis

Sebla B. Kutluay, Daniel Blanco-Melo, Trinity Zang, and Paul D. Bieniasz
The Aaron Diamond AIDS Research Center, Rockefeller University, Howard Hughes Medical Institute

Gag Trafficking, Membrane/Env Interactions, and Assembly

T31. Determining the Role of Nuclear Trafficking of the Retroviral Gag Protein

Rebecca J. Kaddis[1], Breanna L. Rice[1], Estelle Chiari-Fort[1], Timothy Lochmann[1], Matthew Stake[1], Nikoloz Shkriabai[3], Mamuka Kvaratskhelia[3] and Leslie J. Parent[1, 2]
Penn State College of Medicine, [1]Department of Medicine, and [2]Department of Microbiology and Immunology, Hershey, PA 17033 and [3]College of Pharmacy, The Ohio State University, Columbus, OH

T32. Membrane Coupling of HIV-1 MA Domain to Lipid Bilayers: Factors that Influence Affinity and Protein Layer Structure

Hirsh Nanda[1,2], Marilia Barros[1], Siddhartha Datta[3], Alan Rein[3], Mathias Lösche[1,2]
[1]Carnegie Mellon University, Dept of Physics, Pittsburgh, PA; [2]National Institute of Standards and Technology, Center for Neutron Research, Gaithersburg, MD; [3]National Cancer Institute, HIV Drug Resistance Program, Fredrick, MD

T33. Structural Basis for Membrane Targeting by the HIV-1 Matrix Protein

Mercredi, P. Y., Bucca N., Loeliger, B., Gaines C. R., Bhargava, P., Mehta, M., and Summers M. F.
Howard Hughes Medical Institute, Department of Chemistry and Biochemistry, University of Maryland, Baltimore County, Baltimore, MD 21250, USA

T34. Novel Techniques to Study Gag-Membrane Interactions In Vitro

Robert A. Dick[1], Yi Wen[1], Fred Heberle[2], Thais Enoki[1], Rebecca Simpson[1], Milka Doktorova[3], Juan Wang[4], John Katsaras[2], Warren Zipfel[4], Volker M Vogt[1], and Gerald Feigenson[1]
[1]Dept of Molecular Biology and Genetics, Cornell University: [2]Biology and Soft Matter Division, Neutron Sciences Directorate, Oak Ridge National Laboratory; [3]Tri-Institutional Training Program in Computational Biology and Medicine, Weill Cornell Medical College; [4]Department of Biomedical Engineering, Cornell University

T35. Biophysical Studies of RSV Gag Conformation and Membrane Binding.

Robert A Dick[1], Hirsh Nanda[2], Alan Rein[3], Volker M. Vogt[1], Siddhartha Datta[2]
[1]Dept of Molecular Biology and Genetics, Cornell University; [2]NIST Center for Neutron Research, National Institute of Standards and Technology; [3]HIV Drug Resistance Program, National Cancer Institute

T36. HIV-1 Tat Membrane Interaction Probed Using X-Ray and Neutron Scattering, CD Spectroscopy and MD Simulations

Kiyotaka Akabori[1], Kun Huang[2], Bradley W. Treece[1], Michael S. Jablin[1], Brian Maranville[3], John F. Nagle[1], Angel E. Garcia[2], and Stephanie Tristram-Nagle[1]
[1]Biological Physics Group, Department of Physics, Carnegie Mellon University, Pittsburgh, PA; [2]Department of Physics, Rensselaer Polytechnic Institute, Troy, NY; [3]NIST Center for Neutron Research, Gaithersburg, MD

T37. HIV-1 Envelope Glycoprotein Incorporation Requires Matrix Trimerization

Philip R. Tedbury, Sherimay D. Ablan, and Eric O. Freed
HIV Drug Resistance Program, NCI at Frederick, Frederick, MD. USA.

T38. Characterization of Novel Mutations in the HIV-1 Env Glycoprotein that Enhance Cell-to-Cell Viral Transfer

Rachel Van Duyne, Lillian Kuo, Ken Fujii, Eric O. Freed
Virus-Cell Interaction Section, HIV Drug Resistance Program, NCI-Frederick, Frederick, MD

T39. Identification of a Novel Structural Element of Gag Important for the Assembly of Immature HIV-1 Particles

Mariia Novikova, Muthukumar Balasubramaniam, Sagar Kudchodkar, and Eric O. Freed.
Virus-Cell Interaction Section, HIV Drug Resistance Program, NCI-Frederick, Frederick, MD

T40. Elements of Lentiviral p6 Domain Modulating Viral Assembly

Yul Eum Song, and Marc C Johnson
Department of Molecular Microbiology Microbiology and Immunology, University of Missouri-Columbia

T41. PTAP L Domain-Dependent Modulation of Ca^{2+} Signaling Facilitates HIV-1 Gag Assembly and Budding

Lorna S. Ehrlich[1], Gisselle N. Medina[1#], Sara Photiadis[1], Paul B. Whittredge[2], Justin W. Taraska[2], and Carol A. Carter[1]
[1]Molecular Genetics & Microbiology, Stony Brook University, Stony Brook, NY, 11794; [2]Laboratory of Molecular Biophysics, National Heart Lung and Blood Institute, National Institutes of Health, Bethesda, MD 20892; [#]Current Address: PLUM Island Disease Center ARS-USDA, Greenport, NY 11944

T42. Temporal and Spatial Organization of ESCRT Protein Recruitment During HIV-1 Budding

Marina Bleck[1], Michelle S. Itano[1], Daniel S. Johnson[1], Paul D. Bieniasz[2], Sanford M. Simon[1]
[1]Laboratory of Cellular Biophysics, The Rockefeller University, 1230 York Avenue, New York, NY 10065; [2]Howard Hughes Medical Institute, Aaron Diamond AIDS Research Center, Laboratory of Retrovirology, The Rockefeller University, New York, NY 10016.

T43. The Vps4 AAA ATPase Binds ESCRT-III Substrates as an Asymmetric Hexamer

Han Han, Nicole Monroe, Debra M. Eckert, Joerg Votteler, Michael S. Kay, Wesley I. Sundquist, Christopher P. Hill
Department of Biochemistry, University of Utah, Salt Lake City, UT, USA

T44. Molecular Modeling of the Spacer Peptide Region of Immature Retroviral Gag

Boon Chong Goh[1], Juan R. Perilla[1], Katrina J. Heyrana[2], Rebecca C. Craven[2], and Klaus Schulten[1]
[1]Department of Physics and Beckman Institute, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA; [2]Department of Microbiology and Immunology, Penn State University, Hershey, PA 17033, USA

T45. Associative Interactions of the SP1 Domain of HIV-1 Gag

Siddhartha A Datta[1], Patrick Clark[3], Raymond Sowder[3], Marzena Dyba[2], Raul Cachau[3], and Alan Rein[1]
[1]HIV Drug Resistance Program, NCI-Frederick, Frederick, MD, [2]Biophysics Resource in the Structural Biophysics Laboratory, NCIFrederick, Frederick, MD, [3]Frederick National Laboratory for Cancer Research, Leidos Biomedical Inc, Frederick, MD.

Gag/PR Interplay, Co-Evolution, and Maturation

T46. MAS NMR Studies of HIV-1 Maturation Intermediates: Investigation of SP1 Conformation in Tubular Assemblies

Christopher L. Suiter[1,2], Caitlin Quinn[1,2], Guangjin Hou[1,2], Jinwoo Ahn[2,3], In-Ja Byeon[2,3], Sherimay Ablan[4], Eric O. Freed[4], Angela M. Gronenborn[2,3], and Tatyana Polenova[1,2]
[1]Department of Chemistry and Biochemistry, University of Delaware; [2]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine; [3]Department of Structural Biology, University of Pittsburgh School of Medicine; [4]HIV Drug Resistance Program, Center for Cancer Research, National Cancer Institute

T47. HIV-1 Maturation *In Vitro*

Ernest L. Yufenyuy[1], Xiaofeng Fu[2], Peijun Zhang[2], and Christopher Aiken[1]
[1]Department of Pathology, Microbiology and Immunology, Vanderbilt School of Medicine, Nashville, TN 37232, USA; [2]Department of Structural Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260, USA

T48. Characterization of HIV-1 GagΔ Proteolysis in Vitro at the Capsid/SP1 and SP1/Nucleocapsid Cleavage Sites

Jesse Crayle[a], Marc Potempa[b], Sook-Kyung Lee[ac], Ronald Swanstrom[abc].
[a]Department of Biochemistry and Biophysics, [b]Department of Microbiology and Immunology, and the [c]UNC Center for AIDS Research, University of North Carolina at Chapel Hill

T49. Viral Quasispecies and Mutational Analysis Using Next-Generation Sequencing

Andrew Routh[1], Danny Antaki[1], Max Chang[2], Jason F. Okulicz[3], and Bruce Torbett[1]
[1]Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA 92037, USA; [2]Integrative Genomics and Bioinformatics Core, The Salk Institute for Biological Studies, La Jolla, CA 92037, USA; [3]HIV Medical Evaluation Unit, Infectious Disease Service, San Antonio Military Medical Centre, TX 78234, USA

T50. Structural Basis of Drug Resistant I50V/A71V HIV-1 Protease and Gag Substrate Coevolution

Kuan-Hung Lin, Aysegul Ozen, Nese Kurt Yilmaz, and Celia Schiffer
University of Massachusetts Medical School

T51. Deep Sequencing of Many Drug Resistant HIV Patients Reveals Correlated Mutation Patterns in Gag and Protease

William F. Flynn[1,2], Zhiqiang Tan[3], Max W. Chang[4], Glenn Oliveira[4], Jinyun Yuan[4], Jason F. Okulicz[5], Bruce E. Torbett[4], and Ronald M. Levy[2,6]
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T52. Structural Insights into Substrate Co-Evolution

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T53. Probing Molecular Interactions of Protease with Small Molecules and Gag Cleavage Junctions

Tiefenbrunn, T[1]., Forli, S.[1], Happer, M.[2], Gonzalez, A[3]., Baksh, M. M.[4], Chang, M. W.[5], Tsai, Y.-S.[3], Lin, Y.-C.[2], Perryman, A. L.[1], Rhee, J.-K.[4], Torbett, B.E.[5], Olson, A. J.[1], Soltis, M.[3], Elder, J. H.[2], Finn, M. G.[4], Stout, C. D.[1]
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PR Activity, Structure, and Inhibition

T54. Deep Sequencing Analysis Reveals Novel Pathways to Protease Inhibitor Resistance: In Vitro Selection of Resistance Mutations to New Potent HIV-1 Protease Inhibitors

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T55. The Fitness Landscapes of Drug-Resistance in HIV Protease

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T56. Characterizing Conformational Sampling and Dynamics of HIV-1 Protease via EPR Spectroscopy and NMR Spectroscopy

Xi Huang, Lingna Hu, Zhanglong Liu and Gail E. Fanucci
Dept. of Chemistry, University of Florida

T57. REdiii: A Pipeline for Automated Structure Solution

Markus-Frederik Bohn and Celia A Schiffer
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T58. Understanding Molecular Recognition in HIV Protease and Integrase Using Binding Free Energy Methods

Nanjie Deng[1], R. S. K. Vijayan[1], L. Wickstrom,[1] P. He[1], A. Perryman[2], S. Forli,[2] T. Tiefenbrunn,[2] D. Stout,[2] E. Gallicchio,[1] M. Kvaratskhelia,[3] A. J. Olson[2], R. M. Levy[1]
[1]Temple University, Philadelphia; [2]The Scripps Research Institute, La Jolla; [3]Ohio State University

T59. Incorporating Bridging-Waters into Lead Discovery Using Molecular Footprints

Jiaye Guo[1] and Robert C. Rizzo[2,3,4]
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T60. Understanding the Structural Basis of Drug Resistance in Influenza Neuraminidase

Kristina Prachanronarong, Ellen Nalivaika, Aysegul Ozen, Safak Yilmaz, Kelly Thayer, Jennifer Wang, Konstantin Zeldovich, Robert Finberg, and Celia Schiffer
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T61. Examining the Genetic Barrier of GRL008, a Novel HIV-1 Protease Inhibitor Designed Using Crystallographic Solvent Mapping Technique

Ravikiran S. Yedidi[1], Harisha Garimella[1], Manabu Aoki[2,3], Hiromi Aoki-Ogata[2], Darshan V. Desai[1], Simon B. Chang[1], David A. Davis[4], W. Sean Fyvie[5], Joshua D. Kaufman[6], David W. Smith[7], Debananda Das[1], Paul T. Wingfield[6], Kenji Maeda[1], Arun K. Ghosh[5] and Hiroaki Mitsuya[1, 2]

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T62. The Catalytic Activity of the HIV-1 Protease is Enhanced by RNA

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Capsid, Core Assembly, and Cyclophylin

T63. High Resolution Structure of HIV-1 Capsid Assembly

Gongpu Zhao[1,2], Xueming Li[3,4], Chuang Liu[1,2], Jiying Ning[1,2], Randall T. Schirra Jr.[1,2], Angela M. Gronenborn[1,2], Yifan Cheng[3] and Peijun Zhang[1,2]

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T64. Coarse-Grained Molecular Simulations of HIV-1 Capsid Protein Self-Assembly

John M. A. Grime and Gregory A. Voth

Department of Chemistry, James Franck Institute, Institute for Biophysical Dynamics and Computation Institute, University of Chicago, Illinois 60637

T65. Atomistic Insights of the HIV Capsid from Molecular Dynamics Simulations

Juan R. Perilla[1] and Klaus J. Schulten[1]

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T66. Backbone Dynamics in HIV-1 Capsid Protein Assemblies by Hybrid MAS NMR/MD Approach: Insights Into Conformational Plasticity and Interactions with Cyclophilin A

Guangjin Hou[1,2], Huilan Zhang[1,2], Manman Lu[1,2], Christopher L. Suiter[1,2], Suvrajit Maji[3], Christopher J. Langmead[3], Juan R. Perilla[5], Jinwoo Ahn[2,4], In-Ja L. Byeon[2,4], Peijun Zhang[2,4], Klaus Schulten[5], Angela M. Gronenborn[2,4], Tatyana Polenova[1,2]

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T67. Structure of Mature HIV-1 Capsid Assembly in Complex with Cyclophilin A

Chuang Liu[1,2], Juan R. Perilla[3], In-Ja Byeon[1,2], Tatyana Polenova[2,4], Chang-Hyeock Byeon[1,2], Jiying Ning[1,2], Jinwoo Ahn[1,2], Chris Aiken[2,5], Angela M. Gronenborn[1,2], Klaus Schulten[3] and Peijun Zhang[1,2]

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T68. Structural Characterization of HIV-1 Capsid Protein Assemblies Free and in Complex with Cyclophilin A Using Magic Angle Spinning NMR

Manman Lu[1,2], Guangjin Hou[1,2], Huilan Zhang[1,2], Christopher L. Suiter[1,2], Rupal Gupta[1,2], Mingzhang Wang[1,2], Jinwoo Ahn[2,3], Chang Byeon[2,3], In-Ja L. Byeon[2,3], Angela M. Gronenborn[2,3], Tatyana Polenova[1,2]

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Non-Integration Functions of Integrase

T69. An Essential Role of INI1/hSNF5 in HIV-1 Post-Transcriptional Mechanisms Leading To Assembly

AnnaLena La-Porte, Jennifer Cano, Xuhong Wu, Doyal Mitra and Ganjam V. Kalpana

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T70. Inhibition of HIV-1 Particle Maturation by Allosteric Integrase Inhibitors

Kellie A Jurado[1], Juan Fontana[2], James R. Fuchs[3], Mamuka Kvaratskhelia[4], Alasdair C. Steven[2], Alan Engelman[1]

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T71. HIV-1 IN Mutants Defective for INI1/hSNF5 Binding Exhibit Impaired Particle Morphology, Reverse Transcription and Integration

Sheeba Mathew[1], Savita Bhutoria[2], Annalena LaPorte[1], Xuhong Wu[1], Menachem Spira[1], Minh Nguyen[1], Achintya Pal[1], Seetarama Acharya[2] and Ganjam V. Kalpana[1]

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Vpu and Nef

T72. Structural Basis of HIV-1 Vpu-Mediated BST2 Antagonism *via* Hijacking of the Clathrin Adaptor Protein Complex 1

Xiaofei Jia[1], Erin Weber[1], Andrey Tokarev[2], Mary Lewinski[2], Maryan Rizk[2], Marissa Suarez[2], John Guatelli[2], and Yong Xiong[1]

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T73. Structural Basis of Protein-Protein Interactions of Vpu from HIV-1

Stanley J. Opella[1], Hua Zhang[1], Eugene Lin[1], John Guatelli[2], Mary Lewinski[2], Moein Jafari[2], Bharatwaj Sowrirajan[3], and Edward Barker[3]

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T74. Characterization of Vpu-SCF E3 Ligase Complex

Shujun Yuan[1], Martin Lynn[1], Wu Shenping[1], Shenheng Guan[2], Yifan Cheng[1], Robert M. Stroud[1]

[1]Department of Biochemistry and Biophysics, University of California San Francisco, San Francisco, CA; [2]Department of Pharmaceutical Chemistry, University of California San Francisco, San Francisco, CA

T75. How HIV-1 Nef Hijacks the AP-2 Clathrin Adaptor to Downregulate CD4

Xuefeng Ren[1,2,3], Sang Yoon Park[3], Juan S Bonifacino[3], James H Hurley[1,2]

[1]Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley; [2]California Institute for Quantitative Biosciences, University of California, Berkeley, Berkeley; [3]Cell Biology and Metabolism Program, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda.

Friday Posters

Envelope, gp120 and Inhibitors

F1. Antibody Affinity Maturation in an HIV Broadly Neutralizing B-cell Lineage: a Shift in Relative Orientation of Light- and Heavy-Chain Variable Domains

Daniela Fera[a], Aaron G. Schmidt[a], Barton F. Haynes[b], Feng Gao[b], Hua-Xin Liao[b], Thomas B. Kepler[c], and Stephen C. Harrison[a,d]
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F2. Investigations into the Lytic Mechanism of Peptide Triazole Thiols and the Comparison with HIV-1 Fusion

RV Kalyana Sundaram[1], LD Bailey[1], A Rosemary Bastian[1], R Aneja[1], K Weiss[1], J Huynh[1], C Duffy[1], A Holmes[1], A Ahmed[1], JM Jacobson[1], SP Wrenn[1], CF Abrams[1], H Li[2], DRM Moreira[3], A Emileh[4], G Leslie[5], JA Hoxie[5], M Root[6], and IM Chaiken[1]
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F3. Potency Enhancement and Suppression of Infectious Virus Formation Using Multivalent Display of Peptide Triazole HIV-1 Entry Inhibitors on Gold Nanoparticles

A. Rosemary Bastian[1], C. Ang[1], A. Nangarlia[1], Y. H. Huang[1], L. D. Bailey[1], R. V. K. Sundaram[1], K. McFadden[2], F. Shaheen[3], D. R. M. Moreira[4], C. Duffy[1], J. A. Hoxie[3], C. F. Abrams[1], M. Root[5], J. Jacobson[1], and I. Chaiken[1]
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F4. Functional Determinants of Peptide Triazole Dual Receptor Antagonists Utilize Two Conserved Hydrophobic Pockets of HIV-1 gp120

Adel Ahmed, Rachna Aneja, Bibek Parajuli, Lauren Bailey, Huiyuan Li, Ramalingam Venkat Kalyana Sundaram and Irwin Chaiken
Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia PA 19102

F5. Structure-Based Design, Synthesis and Validation of Small Molecule CD4-Mimetic Inhibitors of HIV-1 Entry

Bruno Melillo,[(1)] Joel R. Courter,[(1)] Navid Madani,[(2)] Joseph Sodroski,[(2)] Arne Schön,[(3)] Ernesto Freire,[(3)] Peter D. Kwong,[(4)] Wayne A. Hendrickson,[(5)] Irwin M. Chaiken,[(6)] Judith M. Lalonde,[(7)] and Amos B. Smith, III[(1)]

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F6. Conformational Space Search and Analysis for Carbohydrates

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F7. Understanding Glycan Type Specificity in Highly Glycosylated Proteins

Artem Krantsevich and David F. Green

Stony Brook University

F8. Statistical Analysis of the Glycosylation Sites in HIV-1 Envelope Protein

Nikolay Krantsevich and David F. Green

Stony Brook University

F9. Understanding Dynamic Structural Variations in HIV-1 Envelope Glycoprotein gp120

Tuoling Qiu[1], David F. Green[1,2]

[1]Chemistry Department, Stony Brook University, Stony Brook, NY 11794; [2]Department of Applied Mathematics and Statistics, Stony Brook University, Stony Brook, NY 11794

F10. Conformational Dynamics Of Single HIV-1 Env Trimers On The Surface Of Native Virions

James B. Munro[1], Jason Gorman[2], Xiaochu Ma[1], James Arthos[3], Dennis R. Burton[4,5], Wayne C. Koff[6], Joel R. Courter[7], Amos B. Smith III[7], Peter D. Kwong[2], Scott C. Blanchard[8] & Walther Mothes[1]

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F11. The Structural Stability of gp120 and Trimeric gp140

Arne Schön and Ernesto Freire

Johns Hopkins University, Baltimore, MD 21218

F12. Maximum-Unbiased Benchmarking Datasets for Ligand-Based Drug Discovery targeting Human Chemokine Receptorome

Jie Xia[╟, §], Terry-Elinor Reid[§], Ermias Lemma[§], Eyob Hailu[§], Liangren Zhang[╟], and Xiang Simon Wang[§*]

[╟]State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Beijing 100191, China; [§]Molecular Modeling and Drug Discovery Core for District of Columbia Developmental Center for AIDS Research (DC D-CFAR); Laboratory of Cheminfomatics and Drug Design, Department of Pharmaceutical Sciences, College of Pharmacy, Howard University, Washington DC 20059, U.S.A.

Envelope, gp41 and Inhibitors

F13. The Dependence of HIV Env-Mediated Fusion on Phosphatidylserine in the Target Cell Membrane

Elena Zaitseva[1], Eugene Zaitsev[1], Kamran Melikov[1], Mariana Marin[2], Gregory B. Melikyan[2], Leonid V. Chernomordik[1]

[1]Section on Membrane Biology, NICHD, NIH; [2]Emory University Children's Center, Atlanta, Georgia

F14. Small Molecule Docking Using Property-Based Volume Overlap

Yuchen Zhou[1] and Robert C. Rizzo[1, 2, 3]

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F15. Human Neutrophil Peptide 1 Sensitizes HIV-1 to Antibodies and Inhibitors Targeting Intermediate Conformations of gp41

Lusine Demirkhanyan[1], Mariana Marin[1], Wuyuan Lu[2], and Gregory B. Melikyan[1,3]
[1]Division of Pediatric Infectious Diseases, Emory University Children's Center; [2]Institute of Human Virology, University of Maryland School of Medicine; [3]Children's Healthcare of Atlanta.

F16. gp41 Ectodomain Dissociates and Forms a Stable Monomer on Phospholipid Vesicles and Detergent Micelles: Implication for the HIV-1 Env-Mediated Membrane Fusion

Julien Roche, John M. Louis, Alexander Grishaev, Jinfa Ying and Ad Bax
Laboratories of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892

F17. HIV Resistance Mechanisms to D-Peptide Entry Inhibitors

Amanda Smith, Matthew Weinstock, Frank Whitby, Chris Hill, and Michael Kay
Department of Biochemistry, University of Utah, Salt Lake City, UT

F18. Pharmacophore Matching Similarity Score In DOCK With Application To HIVgp41

Lingling Jiang[1] and Robert C. Rizzo[1,2,3]
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F19. Reverse Hairpin Constructs of the gp41 Ectodomain are Potent Fusion Inhibitors by Virtue of an Exposed Hydrophobic Pocket on the N-Heptad Repeat

Shidong Chu, Hardeep Kaur, Joseph Walsh and Miriam Gochin
Touro University College of Osteopathic Medicine, Vallejo CA 94592

F20. Computer-Aided Design of Small Molecule Inhibitors of HIVgp41

Brian C. Fochtman[1] and Robert C. Rizzo[2,3,4]
[1]Department of Biochemistry and Cellular Biology, [2]Department of Applied Mathematics & Statistics, [3]Institute of Chemical Biology & Drug Discovery, [4]Laufer Center for Physical & Quantitative Biology, Stony Brook University, Stony Brook, NY 11794

F21. Small Molecule Inhibition of HIV gp41 NHR Trimer Formation

William J. Allen,[1] Hyun Ah Yi,[2] Miriam Gochin,[3,4] Amy Jacobs,[2] and Robert C. Rizzo[1,5,6]
[1]Department of Applied Mathematics & Statistics, Stony Brook University, Stony Brook, New York 11794, [2]Department of Microbiology and Immunology, State University of New York at Buffalo, Buffalo, New York 14214, [3]Department of Basic Sciences, Touro University—California, Mare Island, Vallejo, California 94592, [4]Department of Pharmaceutical Chemistry, University of California San Francisco, San Francisco, California 94143, [5]Institute of Chemical Biology & Drug Discovery, Stony Brook University, Stony Brook, New York 11794, [6]Laufer Center for Physical & Quantitative Biology, Stony Brook University, Stony Brook, New York 11794.

Trim5α Structure and Function

F22. The Tripartite Motif Coiled-Coil Is an Elongated Antiparallel Hairpin Dimer

Jacint G. Sanchez[a,1], Katarzyna Okreglicka[a,1], Viswanathan Chandrasekaran[b], Jordan M. Welker[a], Wesley I. Sundquist[b], and Owen Pornillos[a,2]
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F23. Hydrogen/Deuterium Exchange Studies of TRIM Family Proteins

Dewey Brooke[1], Brady Summers[2] , Yong Xiong[2], Jinwoo Ahn[3], and Peter Prevelige[1]
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F24. The Role of the SPRY Domain V1 Loop Flexibility in the Restriction Activity of TRIM5alpha.

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University of Texas Health Science Center at San Antonio, San Antonio, TX 78229

F25. Alpha-Helices in the Coiled-Coil Linker Region Govern Rhesus TRIM5α Assembly and Restriction

Jaya Sastri[1,4], Santanu Mukherjee[2], Rajan Lamichhane, Nikolai Smolin[3], Seth Robia[3], David P. Millar, and Edward M. Campbell[1,2,3]
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F26. Electron Microscopic and Biochemical Characterization of Recombinant TRIM5α Proteins and Their Capsid Complexes

Yen-Li Li[1], Viswanathan Chandrasekaran[1], Stephen D. Carter[2], Barbie Ganser-Pornillos[3,4], Cora L. Woodward[2], John E. Heuser[5], Mark Yeager[3,4], Grant J. Jensen[2,6], Wesley I. Sundquist[1]
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F27. Correlated Cryogenic Light and Electron Microscopy to Visualize Cytoplasmic TRIM5α Bodies

Stephen D. Carter[1], Cora L. Woodward[1] Yen-Li Li[2], Viswanathan Chandrasekaran[2], Barbie Ganser-Pornillos[4,5], Thomas J. Hope[7], John E. Heuser[6], Mark J. Yeager[4,5], Wesley I. Sundquist[2], and Grant J. Jensen[1,2]

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RT Structure, Function, and Inhibition

F28. Another Connection Between Capsid and Reverse Transcription

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F29. A Structure-Based Mechanism for tRNA and Retroviral RNA Remodeling During Primer Annealing

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F30. SAXS Analysis Reveals Conservation of tRNA Structural Mimicry in the HIV-1 5´UTR and Model of the RT Initiation Complex

William A Cantara[1], Erik D Olson[1], Christopher P Jones[1], Roopa Comandur[1], William Ho[2], Steven Tuske[2], Eddy Arnold[2], and Karin Musier-Forsyth[1]

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F31. RNA Structural Regulation of Reverse Transcription Initiation in HIV

Aaron T. Coey, Margreth Mpossi, Joseph Puglisi and Elisabetta Viani Puglisi

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F32. Ty3 Reverse Transcriptase Complexed with an RNA-DNA Hybrid Shows Structural and Functional Asymmetry

Nowak, E[1], Miller, JT[2], Bona, MK[2.3], Studnicka[1], J, Szczepanowski[4], RH, Jurkowski, J[1], Le Grice, SFJ[2] and Nowotny, M[1]

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F33. Mismatch Resolution of HIV-1 WT and AZT-Resistant Reverse Transcriptase Using a Forced Copy Choice Recombination System

Siarhei Kharytonchyk, Steven R. King, Clement Ndongmo, Krista L. Stilger, and Alice Telesnitsky
Department of Microbiology & Immunology, University of Michigan, MI 48109, USA

F34. Structural Insights into RT Mutation Q151M that Confers Multi-Nucleoside Drug Resistance

Sergio E. Martinez, Kalyan Das, and Eddy Arnold
Center for Advanced Biotechnology and Medicine, Department of Chemistry and Chemical Biology, Rutgers University, Piscataway, New Jersey 08854, USA.

F35. Rilpivirine Inhibition of HIV-1 RT Initiation: A Pre-Steady State Kinetic Approach

Kamalendra Singh[1,2], Jacqueline A. Flores[1,2], Briana M. Bester[1,2], Obiaara B. Ihenacho[1,2], Zhe Li[1,3], Steve Tuske[4], Kalyan Das[4], Michael A. Parniak[5], Eddy Arnold[4], and Stefan G. Sarafianos[1,2,3]
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F36. Structural Studies on Understanding and Overcoming NRTIs' Drug Toxicity

Michal Szymanski, Vladmir Kuznetsov, Christie Shumate and Whitney Yin
Dept. Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX 77555

F37. Structural Integrity of the Ribonuclease H Domain in HIV-1 Reverse Transcriptase

Ryan L. Slack[1], Justin Spiriti[2], Michael A. Parniak[3], Daniel M. Zuckerman[2], and Rieko Ishima[1]
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Vif, APOBECs, and Their Inhibition

F38. Atomic Force Microscopy to Elucidate Sequence Context Dependent Interaction of APOBEC3G with ssDNA

Jaspreet Banga[1], Luda S. Shlyakhtenko[1], Alexander Lushnikov[1], Ming Li[2], Reuben S. Harris[2] and Yuri L Lyubchenko[1]
[1]Department of Pharmaceutical Sciences, College of Pharmacy, University of Nebraska Medical Center, Omaha, NE 68198; [2]Department of Biochemistry, Molecular Biology, and Biophysics, Institute for Molecular Virology, Center for Genome Engineering, Masonic Cancer Center, University of Minnesota, 321 Church Street S.E., 6-155 Jackson Hall, Minneapolis, MN 55455.

F39. Small Molecule Probes of APOBEC3-Catalyzed Mutation

Margaret E. Olson[1], Angela L. Perkins[1], Ming Li[2], Reuben S. Harris[2], Daniel A. Harki[1]
[1]Department of Medicinal Chemistry, University of Minnesota, Minneapolis, MN 55455; [2]Department of Biochemistry, Molecular Biology & Biophysics, Institute for Molecular Virology, University of Minnesota, Minneapolis, MN 55455

F40. Small Molecule APOBEC3G Activators as a Novel Strategy for the Prevention of HIV Infection

Kimberly M. Prohaska and Harold C. Smith
OyaGen, Inc. Rochester, NY

F41. The Dinucleotide Preference of APOBEC3G Is Dispensable for HIV-1 Restriction

Anurag Rathore, Michael A. Carpenter, Terumasa Ikeda, Ming Li, Nadine Shaban, Emily Law, and Reuben S. Harris
Department of Biochemistry, Molecular Biology, and Biophysics, Institute for Molecular Virology, University of Minnesota, Minneapolis, MN 55455

F42. Inhibition of APOBEC3G Deaminase by Synthetic Peptides Sensitizes Lymphoma Cells to Genotoxic Agents

Prabhu Ponnandy[1], Elena Britan-Rosich[1], Markus F. Bohn[2], Shivender Shandilya[2], Celia Schiffer[2], and Moshe Kotler[1]
[1]The Hebrew University Hadassah Medical School, Jerusalem 91120, Israel; [2]University of Massachusetts Medical School, Worcester, MA 01545, USA

F43. Cross-Species Analysis Reveals Differential Composition of the APOBEC3-Degrading Lentiviral Vif E3 Ligase Complex

Judd F Hultquist[1,3,4], Joshua R Kane[1], Jennifer M Binning[2], David J Stanley[2], Jeffrey R Johnson[1,4], Tasha Johnson[1], Billy Newton[1], Kathy Franks-Skiba[1], Reuben S Harris[3], John D Gross[2], and Nevan J Krogan[1,4]
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F44. HIV-1 Vif Adaptation to Natural APOBEC3H Variants

Marcel Ooms[1], Thijs Booiman[2], Michael Letko[1], Neeltje Kootstra[2], and Viviana Simon[1]
[1]Icahn School of Medicine at Mount Sinai School of Medicine, New York, NY; [2]Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

F45. Species Specific Requirements for Lentivirus Vif Hijacked Ubiquitin Ligase Complex

Jennifer M. Binning[1], Josh Kane[2], Judd F. Hultquist[2], Ming Li[3], Rueben S. Harris[3], Nevan J. Krogan[2], and John D. Gross[1]
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F46. Towards Comprehensive Sequence-Function Maps for Vif

Dan Bolon
University of Massachusetts Medical School, Worcester, MA

F47. Exploiting Viral-Host Variation to Define the HIV-1 Vif-A3G Interface

Michael Letko[1,2], Viviana Simon[1,2,3], Marcel Ooms[1,2]
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F48. Viral Evolution and Directed Mutagenesis Studies Inform a Model for the Interaction of Human APOBEC3F with HIV-1 Vif

John S. Albin[1], Elizabeth M. Luengas[1], Allison M. Land[1], Christopher Richards[1,2,3], Brett D. Anderson[1], Nadine M. Shaban[1], Ozlem Demir[4], John R. Holten[1], John S. Anderson[1], Daniel A. Harki[2,3], Rommie E. Amaro[4] and Reuben S. Harris[1,2]
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F49. Novel HIV Drug Screening Targeting the Interaction Between HIV-1 Vif and CBFβ

Ryan P. Bennett and Harold C. Smith
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F50. In Silico Molecular Screening of the HIV-1 Vif Protein to Enable Drug Discovery

Jason D. Salter, Guillermo Morales, and Harold C. Smith
OyaGen, Inc., Rochester, NY

F51. Fab Assisted Electron Microscopy of HIV Vif Complexes

Hai Ta, Natalia Sevillano, Shenping Wu, Dong Young Kim, David Paquette, Yifan Cheng, Charles Craik and John Gross
UCSF

F52. Ubiquitin Regulation of ZAP-70 Activity and Modulation by HIV Infection

K. Aurelia Ball, Jeffrey R. Johnson, Arthur Weiss, Nevan J. Krogan, and Matthew P. Jacobson
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SAMHD1, Vpr, and Vpx

F53. Novel Structural Regulation for Substrates-Controlled GTP-Activated SAMHD1 Deoxynucleoside Triphosphate Triphosphohydrolase

Chunfeng Zhu[1], Wei Wei[1,2,3], Yong Gong[1,3], and Xiao-Fang Yu[1,2,3]
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F54. Binding of HIV-1 Vpr to the Human Homolog of the Yeast DNA Repair Protein RAD23 (hHR23A) Requires its XPC Binding (XPCB) as well as the Ubiquitin Associated 2 (UBA2) Domains

In-Ja L. Byeon, Jinwon Jung, Maria DeLucia, Leonardus M. I. Koharudin, Jinwoo Ahn, and Angela M. Gronenborn
Department of Structural Biology and Pittsburgh Center for HIV-Host Protein Interactions, University of Pittsburgh, School of Medicine, Pittsburgh, PA, 15261

F55. Elucidating the Evolutionary History of Lentiviral Vpr And Vpx Interactions with Host Proteins

Oliver I Fregoso and Michael Emerman
Fred Hutchinson Cancer Research Center, Human Biology, Seattle, WA

HIV Interactome and New Innate Immune Factors

F56. GPS-Prot: A Visual Explorer for HIV and Human Protein Interactions

Melanie B. Brewer[1,2], Gurupartap Davis[1], Alex Shapiro[3], and Alan Frankel[1,2]
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F57. HINT: Uncovering the Early Immune Response to HIV-1 Infection

Monika Schneider[#], Lars Pache[#], John Young[*], Sumit Chanda[#], Frederic Bushman[^], Michael David[&], Ana Fernandez-Sesma[+], Adolfo Garcia-Sastre[+], Alexander Hoffman[&], Nevan Krogan[>], Viviana Simon[+], Leor Weinberger[>], Steven Wolinsky[%]
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F58. PQBP-1 is an Innate Receptor for HIV-1

Sunnie M. Yoh*, Monika Schneider*, Stephen Soonthornvacharin*, Rana E. Akleh*, Kevin C. Olivieri*, Paul De Jesus*, Chunhai Ruan[#], Elisa de Castro^, Pedro A. Ruiz*, Adolfo Garcia-Sastre^, Sumit K. Chanda*
**Infectious and Inflammatory Disease Center, Sanford Burnham Medical Research Institute; [#]Department of Chemistry, University of Michigan; ^Department of Microbiology, Icahn School of Medicine at Mount Sinai*

F59. Identification of Host Cell Mechanisms Restricting HIV-1 Replication

Lars Pache[1], John Marlett[2], Ana Maestre[3], Lara Manganaro[3], Jeff Murry[2], Young Hwang[4], Kevin Olivieri[1], Arturo Diaz[2], Paul De Jesus[1], Crystal Shih[1], Renate König[1,5], Frederic D Bushman[4], Viviana Simon[3], Ana Fernandez-Sesma[3], John A Young[2] and Sumit K Chanda[1]

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BACK

RNA Structures and Protein Interactions Involved in HIV Splicing

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Alternative splicing is a key event of the HIV replication cycle; however, little is known about the RNA structures and protein interactions that regulate splice site selection. Acceptor site A7 is one of the better-characterized sites, where its activity along with donor site D4 is required to remove the Rev Responsive Element and produce multiply spliced transcripts encoding *tat, rev,* and *nef*. The activity of A7 is suppressed by an intronic splicing silencer (ISS), a bipartite exonic splicing silencer (ESS3a/b), and activated by an exonic splicing enhancer (ESE3). The hnRNP A1 protein binds the silencer elements to effectively block SRSF1-ESE3 interaction, thereby inhibiting A7 usage. The series of molecular events that result in the competitive displacement of SRSF1 by hnRNP A1 remain poorly defined, however. To gain insight into the molecular mechanisms that regulate ssA7, we have investigated the structural, biophysical, and biochemical basis of hnRNP A1 recognition of the ISS and ESS3 elements. Here, we present solution NMR structures of these RNA elements and described their binding mechanisms to hnRNP A1. The collective results provide the first insights into how hnRNP A1 binds HIV RNA stem loop structures.

BACK

Global Identification of the RNA Targets of HIV-1 Gag During Virion Genesis

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The Aaron Diamond AIDS Research Center, Rockefeller University, Howard Hughes Medical Institute

HIV-1 Gag protein selectively packages two copies of the viral genome, an event thought to be primarily mediated by interactions between the packaging signal (Ψ) on the viral genome and the nucleocapsid domain of Gag. However, the Ψ sequence is defined based primarily on genetic studies and limited in vitro binding data. To date, no assay has been able to demonstrate a direct and specific interaction between Ψ and Gag protein in a relevant context, i.e. in live cells and in virions. Importantly, deletion of Ψ does not completely abolish genome encapsidation, suggesting that packaging may be more complex than Ψ:Gag interaction, and other regions on the viral genome may contribute. In addition, Gag undergoes several changes in localization, multimerization state and is proteolytically processed during particle genesis, but it is completely unknown how these changes affect RNA-binding properties of Gag. Finally, based on in vitro studies, the matrix (MA) domain of Gag has also been suggested to bind viral and/or cellular RNAs, yet the relevance of this finding has never been tested in a cell-based system. Therefore, we set out to determine globally and at near-nucleotide resolution the RNA targets of Gag during various stages of virion genesis, in cells and in virus particles.

To identify the RNA targets of Gag, we adapted a CLIP-seq (crosslinking-immunoprecipitation-sequencing) methodology, which combines immunoprecipitation of covalently crosslinked protein-RNA complexes with high-throughput sequencing. This method globally identifies the RNA molecules associated with an RNA-binding protein of interest in biological settings and provides near-nucleotide-resolution information about the protein-RNA interaction sites. In addition to determining precisely where within Ψ Gag is bound, we identified several novel sites on the viral genome that are specifically bound by Gag in the cytosol of cells. Mutation of these sites delayed virus replication and we are currently investigating whether this is due to defects in genome packaging. Experiments performed on fractionated cells, mature and immature virions indicated that there are major changes in RNA binding specificity during particle morphogenesis. Surprisingly, even though the MA domain of Gag did not bind viral RNA, a few distinct classes of tRNAs were specifically bound by MA in the cytosol, which appears to play a role in regulating the association of MA with the plasma membrane. Finally, we have also globally identified several cellular RNA molecules, such as 7SL RNA, that are specifically bound by Gag in the cytosol and encapsidated into virus particles.

Our studies indicate that Gag selects viral genome for packaging by binding to a few distinct regions on the viral genome and that association with the plasma membrane and particle formation induces significant changes in the RNA-binding properties of Gag. These studies provide the first dynamic, quantitative and high-resolution picture of viral/cellular RNA interactions with Gag in relevant biological contexts.

BACK

NMR Structure of the HIV-1 Core Encapsidation Signal

Sarah Keane[1], Xiao Heng[1], Jan Marchant[1], Kun Lu[1], Lianko Garyu[1], Sarah Monti[1], Shawn Barton[1], Courtney LaCotti[1], Bilguujin Dorjsuren[1], Gowry Kulandaivel[1], Sai Divakaruni[1], Kedy Edme[1], Simonne Jones[1], Atheeth Hiremath[1], Daniel Tummillo[1], Azra Hosic[1], Siarhei Kharytonchyk[3], Bruce Johnson[1,2], Alice Telesnitsky[3], and Michael F. Summers[1]

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The 5´-leader of the HIV-1 genome directs the selection and packaging of two copies of the unspliced viral RNA into assembling virions. Using a suite of ^{2}H-edited NMR methods, including a novel fragmentation-based ^{2}H-edited approach, we have determined the structure of a 156-nucleotide 5´-leader RNA element that binds the cognate nucleocapsid (NC) protein with high affinity and is independently capable of directing RNA packaging into virus-like particles (Core Encapsidation Signal, Ψ^{CES}). The RNA adopts an unexpected secondary structure that differs considerably from models (more than 20) proposed on the basis of chemical and enzymatic probing. Residues important for splicing and translation are sequestered by base pairing within the core of the RNA, and clusters of unpaired “junction guanosines” are maintained in partially exposed conformations, apparently to promote high affinity NC binding. Long-range Adenosine Interaction Detection (lr-AID) NMR experiments indicate that the structure observed for the isolated Ψ^{CES} RNA also exists in the context of the full-length, 712 nucleotide dimeric 5´-leader. The structure reveals how splicing is attenuated, and dimerization and Gag binding are promoted, by the RNA conformer that directs genome packaging. Progress toward the 3D structure determination of the intact, 712 nucleotide dimeric 5´-leader will be presented.

BACK

Progress Update from the Pittsburgh Center for HIV Protein Interactions

Angela M. Gronenborn

Department of Structural Biology, University of Pittsburgh School of Medicine

The Pittsburgh Center for HIV-Protein Interactions (PCHPI) is focused on understanding the interaction of host proteins with HIV-1 during the so-called early stages of HIV-1 infection, those that occur after viral membrane fusion and prior to integration. During the past year, PCHPI members made significant progress toward understanding the structure and function of TNPO3 (Maertens et al, 2014, PNAS 111(7):2728), the structure of SP1 in CA-SP1 assemblies (Han et al, 2013, J Am Chem Soc 135(47):17793), the structure of SAMHD1 (Ji et al, 2013, Nat Struct Mol Biol 20(11):1304) and a related protein (EF1143, Vorontsov et al, 2014, J Biol Chem 289(5):2815), and the interaction between SAMHD1 and viral proteins Vpr and Vpx (Fregoso et al, 2013, PLOS Pathog 9(7):e10003496; DeLucia et al, 2013, J Biol Chem 288(26):19116). Members of the PCHPI also carried out a structural analysis of APOBEC3A and its DNA binding properties (Mitra et al, 2014 Nucleic Acids Res 42(2):1095) and an analysis of Vpr binding to hHR23A (Jung et al, 2014, J Biol Chem 289(5):2577). Finally, the center has continued its effort to develop imaging methods to follow HIV-1 uncoating (Xu et al, 2013, Retrovirology 10:70) and to develop solid-state NMR for analyzing assembled Capsid (Suiter et al, 2014, J Biolmol NMR, doi:10.1007/s10858-014-9824-4; Hou et al, Isr J Chem 54 (1-2):171). We will briefly summarize the major findings from our studies, highlighting our structural studies of MxB and the assembled Capsid and our recent efforts to image HIV-1 components in cells.

Magic Angle Spinning NMR of HIV-1 Protein Assemblies: Insights into Structure and Dynamics

Guangjin Hou[1,3], Huilan Zhang[1,3], Manman Lu[1,3], Christopher L. Suiter[1,3], Rupal Gupta[1,3], In-Ja L. Byeon[2,3], Jinwoo Ahn[2,3], Angela M. Gronenborn[2,3], and Tatyana Polenova[1,3]

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I will discuss the recent progress from PCHPI in the structural and dynamics characterization of HIV-1 protein assemblies by magic angle spinning (MAS) NMR spectroscopy. MAS NMR is ideally suited for characterization of assemblies of CA, maturation intermediates, and Gag cleavage products since, since it yields atomic-resolution information, does not require cryogenic temperatures and is operational at a wide range of sample conditions, including physiological or close to physiological temperatures and pH. I will demonstrate that these assemblies yield high-resolution MAS NMR spectra permitting in-depth studies of their structure and dynamics. I will discuss the role of structural plasticity and conformational dynamics occurring on timescales from nano- to milliseconds, in the capsid assembly and interactions with the host cell factor Cyclophilin A (CypA). I will describe an integrated NMR and molecular dynamics approach that yielded in-depth understanding of the internal mobility of the assembled CA. I will present our findings on the structure of maturation intermediate CA-SP1 in the assembled state.

Confocal and Super Resolution Imaging of HIV-1 Uncoating and Nuclear Entry

Callen Wallace[1], Hongzhan Xu[2], Christopher Kline[2], Zhou Zhong[2], Doug Fischer[2], Gregory Gibson[1], Simon Watkins[1], and Zandrea Ambrose[2]

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HIV-1 uncoating is a highly controlled process that depends on proper capsid stability and its interaction with multiple host factors, such as cyclophilin A. Premature or delayed dissociation of capsid reduces production and nuclear import of viral DNA, resulting in abortive infection. Visualization of HIV-1 uncoating *in vivo* using microscopy techniques is challenging, mainly due to the dynamic process itself and inefficient labeling of viral proteins and genomes during early infection events. We have fluorescently labeled both the viral capsid and the pre-integration complex (PIC) to visualize dissociation of the core and viral DNA association with host cell factors, such as TNPO3 and nuclear pore proteins, using live cell microscopy and high resolution structured illumination microscopy (SIM). These methods show that wild-type PICs move through the cytoplasm, dock at nuclear pores, and enter the nucleus, while capsid docks at nuclear pores but does not appear to enter the nucleus. HIV-1 core and PIC trafficking is altered in cells in the presence of the capsid inhibitor PF74 or if expression of host cell proteins, such as TNPO3, are depleted. We are currently using these methods with fluorescently tagged cellular proteins and different cell types to understand where uncoating occurs and how wild-type and mutant HIV-1 capsids and PICs interact with host cell pathways and complexes *in vivo*.

BACK

Structural Insight into HIV Restriction by Myxovirus Resistance Protein 2 (MxB)

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The myxovirus resistance (Mx) proteins are interferon-inducible inhibitors of a variety of RNA and DNA viruses. The well-characterized human MxA protein plays a critical role in the innate immune restriction of influenza-like viruses. The related MxB protein has recently been revealed as an interferon-induced restriction factor of HIV-1. MxB's anti-HIV activity occurs early in the viral lifecycle, between reverse transcription of the viral RNA genome and integration of the viral DNA. The nuclear localization signal of MxB is critical for HIV restriction, indicting antiviral activity occurs in or around the nucleus. Interestingly, and contrary to MxA's mode of restriction, MxB mutants that are unable to hydrolyze GTP retain their ability to restrict HIV-1. Specific mutations on the HIV-1 capsid protein (CA) abolish restriction by MxB. The exact step of viral life cycle inhibited by MxB and the inhibition mechanism remain to be elucidated.

To provide insight into the HIV restriction mechanism of MxB we performed detailed biochemical, structural and functional studies. We have determined the crystal structure of nucleotide-free MxB. The structure reveals that MXB adopts an extended conformation similar to that of MxA. The structure is composed of 3 domains, a GTPase domain, a bundle signaling element (BSE) domain that connects and transmits signals between domains, and a carboxy terminal stalk domain that is critical for oligomerization. Two MxB monomers form a dimer in an antiparallel conformation. This structure, in combination with our detailed mutagenesis studies, identifies regions of MxB that are important for HIV restriction. Our results also reveal differences between MxA and MxB, providing insight into how these similar proteins evolved to specifically antagonize different viruses.

BACK

HIVE Center: HIV Interactions and Viral Evolution of Drug Resistance

Eddy Arnold[1], John Elder[2], Alan Engelman[3], M.G. Finn[4], Valery Fokin[5], Patrick Griffin[6], Stephen Hughes[7], Roger Jones[1], Mamuka Kvaratskhelia[8], Ron Levy[1], Joe Marcotrigiano[1], Arthur Olson[9], Michael Parniak[10], Alan Rein[7], Dave Stout[9], and Bruce Torbett[11]

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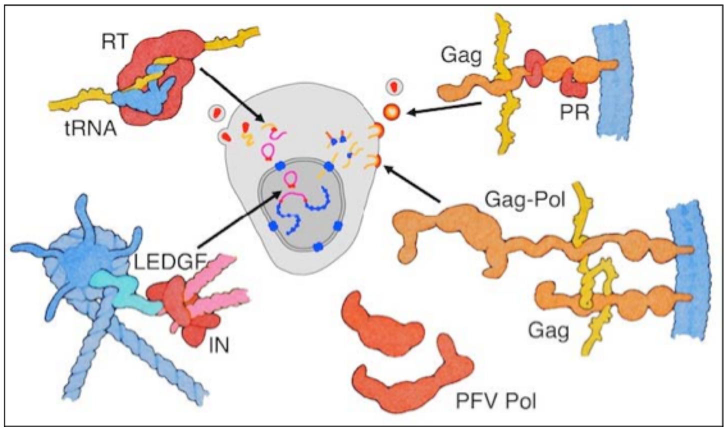

Systems under study in the HIVE Center

The HIVE Center is focused on the structural and dynamic interactions of the major HIV enzymes, reverse transcriptase/RNAse H, protease and integrase, with their molecular partners and effectors in key processes of the viral life cycle. By studying how the structures of the HIV polyprotein precursors direct assembly, maturation and replication, as well as how HIV-Host interactions drive DNA replication and integration, we explore how therapeutic targeting impacts the evolution of drug resistance and what the structural and dynamic consequences of resistance mutations are on the HIV life cycle. This approach is significant because of the promise of new structural insights into the interdependence of viral mechanisms and the potential for new drug design methodologies and therapeutic strategies.

This overview will present the nature of the collaborations and research within the HIVE Center, with selected examples of work that highlight the connections between experimental and computational approaches to understanding HIV structures and the evolution of drug resistance.

A New Class of Multimerization Selective Inhibitors of HIV-1 Integrase

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The quinoline-based allosteric HIV-1 integrase (IN) inhibitors (ALLINIs) are promising candidates for clinically useful antiviral agents. Studies using these compounds have highlighted the role of IN in both early and late stages of virus replication. However, dissecting the exact mechanism of action of the quinoline-based ALLINIs has been complicated by the multifunctional nature of these inhibitors because they both inhibit IN binding with its cofactor LEDGF/p75 and promote aberrant IN multimerization with similar potencies *in vitro*. We have designed small molecules that allowed us to probe the role of HIV-1 IN multimerization independently from IN-LEDGF/p75 interactions in infected cells. We altered the rigid quinoline moiety in ALLINIs and designed pyridine-based molecules with a rotatable single bond to allow these compounds to bridge between interacting IN subunits optimally and promote oligomerization. The most potent pyridine-based inhibitor, KF116, potently (EC_{50} of 0.024 μM) blocked HIV-1 replication by inducing aberrant IN multimerization in virus particles, whereas it was not effective when added to target cells. A genome-wide HIV-1 integration site analysis demonstrated that addition of KF116 to target or producer cells did not affect LEDGF/p75-dependent HIV-1 integration in host chromosomes, indicating that this compound is not detectably inhibiting IN-LEDGF/p75 binding. These findings delineate the significance of correctly ordered IN structure for HIV-1 particle morphogenesis and demonstrate feasibility of exploiting IN multimerization as a therapeutic target.

While crystal structures have shown that ALLINIs bind at the IN catalytic core domain (CCD) dimer interface and bridge two interacting subunits, how these interactions promote aberrant, higher order oligomerization of the protein is not yet known. We used hydrogen-deuterium exchange (HDX) to analyze the multimerization of HIV-1 INs in the presence of the ALLINI inhibitor. The protections were observed in the CCD regions that correlated closely with the inhibitor binding sites seen in our x-ray crystal structures. However, the HDX analyses have also revealed additional protections of the C-terminal domain (CTD) regions that extend beyond the direct inhibitor interacting interface. Consistent with these observations, mass spectrometry-based protein footprinting of surface accessible lysines have revealed inhibitor induced protections of K264 and K266 in full length IN. The K264A/K266A mutant was remarkably resistant to the ALLINI indicted multimerization. Taken together, our studies have revealed the protein-protein interactions that extend beyond the direct inhibitor binding site and lead to aberrant IN multimerization. These studies further our understanding of HIV-1 IN multimerization as a therapeutic target and will facilitate development of improved inhibitors.

BACK

Deep Sequencing of Protease Inhibitor Resistant HIV Reveals Patterns of Mutations in Gag

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Resistance development to HIV inhibitors has contributed to the persistence of HIV/AIDS. While the role of drug resistance mutations in protease has been extensively studied from individuals failing protease inhibitor treatment, mutations in its substrate, Gag, have not been sufficiently investigated. The application of next-generation sequencing has transformed our understanding of the mutational patterns of human viruses. To investigate the extent of changes in HIV protease and Gag in 93 patients undergoing anti-retroviral treatment, serum or plasma samples were obtained when therapy failed to adequately suppress viral replication (~1,000 copies/mL), allowing multiple samples to be obtained from patients that failed once or multiple times.

To reduce the effect of resampling, we maximized cDNA production and usage by adopting a 1-step RT-PCR approach to generate two 1-kb amplicons that spanned Gag and protease. This single round of PCR used 40 cycles and was sufficient to generate appropriate product for >95% of RNA samples. For three clinical samples, we obtained multiple aliquots that were processed independently throughout the entire process of preparation and sequencing. In each case, the paired replicates showed SNP frequencies that were highly correlated. The difference between replicates appeared smallest for the sample with greatest viral load, indicating that a higher number of template molecules can reduce stochastic effects, as might be expected. RT-RCR products were sequence on an Illumina HiSeq, the majority of the reads were mapped to a consensus B reference sequence, generating more that 100,000-fold sequence coverage. The significant sequence coverage within each sample allows the mutational frequency at each position to be determined with precision.

We found that mutations in matrix, capsid, p6, and protease are strongly correlated and often appear in patients who have failed multiple drug therapies. Moreover, many of the novel mutations uncovered in Gag, specifically in matrix, capsid, p1 and p6, occurred outside of the cleavage sites. Additionally, several protease and Gag mutations occur in response to specific protease inhibitor treatments. Identifying patterns of mutations in Gag that are associated with known resistance mutations and/or are associated with therapy failure may provide insights into Gag domain structural relationships, as well as inform future maturation inhibitor design, or future regimen choices for clinicians.

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BACK

A Novel Method for Probing Structural Dynamics of HIV-1 Reverse Transcriptase Interactions with Inhibitors and Nucleic Acids by Hydrogen-Deuterium Exchange Coupled to LC-MS.

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Hydrogen-deuterium exchange coupled to liquid chromatography and mass spectrometry (HDX) is a powerful technique for probing the behavior of proteins in solution. Protein-protein interactions, antibody epitope mapping, and interactions between proteins and their ligands, antagonists, and agonists have all been characterized by HDX. HIV-1 reverse transcriptase (RT) is a multifunctional asymmetric heterodimer composed of 66 kDa (p66) and 51 kDa (p51) subunits. RT binds a variety of nucleic acids, small molecule substrates, and inhibitors; large conformational changes are known to accompany these binding events making it a good subject for study by HDX. However, because p51 shares 440 amino acids with p66, but adopts a different overall conformation it is not possible to unambiguously identify peptides generated from the shared sequence. Also, the presence of different solvent environments for peptides derived from different subunits confounds interpretation. Previous studies on RT dynamics and allosteric effects of RT interaction with the NNRTI efavirenz (EFV) employed selective biotinylation of either subunit to separate RT into p51 and p66 after labeling with deuterium, but before LC-MS analysis (Seckler *et al*., 2009 & 2011).

We have developed a new approach that relies on ^{15}N-labeling of one subunit of RT followed by reconstitution and purification of heterolabeled RT for subsequent HDX analysis. The program MASCOT was modified to identify ^{15}N-labeled peptides from MS/MS data. We performed HDX on RT in the presence and absence of the NNRTI EFV, and in the presence and absence of double-stranded oligomeric DNA, and RNA designed to mimic the HIV-1 initiation complex. Our HDX results with RT and EFV are comparable to those previously reported. The effects on the exchange rate of RT with and without nucleic acids were compared with well-established RT-DNA contacts known from crystal structures. Overall duplex DNA binding to RT results in decreased exchange indicating stabilization of RT relative to its unbound state. The strongest regions of decreased deuterium exchange occurred near the RNase H site (including the RNase H primer grip), the p66 thumb subdomain, and the template grip of the p66 fingers subdomain. These results are consistent with interactions observed with RT-DNA crystal structures. HDX with RT in the presence of RNA duplex containing the PBS and anti-PBS sequence of tRNA showed interactions similar to those observed with DNA but reduced in terms of the total number of deuterons retained for each peptide suggesting RT interactions with RNA are weaker than with DNA.

BACK

Crystal Structure of the Native Hexameric Full-Length HIV-1 Capsid

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The HIV-1 full length capsid protein (CA-FL) is viewed as an attractive therapeutic target since proper capsid formation is required for virus infectivity. CA-FL is generated by Gag processing during the HIV-1 maturation process. CA-FL molecules assemble to form hexamers and pentamers that rearrange into a fullerene cone-shaped structure, containing a ribonucleoprotein complex of the genomic RNA with nucleocapsid at the center of the mature virus. Crystal structures of the native unassembled hexameric CA-FL (without cross-linked residues that might prevent changes in inter- or intra-subunit interactions) are of great interest, as they may provide insights relevant to the development of drugs that prevent or impede the transition from the preassembled to the assembled capsid states.

We have solved the crystal structure of the first hexameric HIV-1 CA-FL in its native form (without engineered cross-linking cysteines and including all residues at the hexamer-hexamer interface) in hydrated and dehydrated forms. There is one molecule per asymmetric unit in each structure, and the P6 space group generates the native hexameric assemblies. Experimental electron density for the following areas provides important information: i) N-terminal domain (CA_{NTD}) residues 4–10 that form the 6-fold symmetric CA_{NTD}-CA_{NTD} interface and stabilize the assembled hexameric state; ii) CA_{NTD} loop residues 85–95 where cyclophilin A (CypA) binds and plays an important role in HIV-1 infectivity; iii) C-terminal domain (CA_{CTD}) residues 176–187 responsible for a 2-fold homodimeric CA_{CTD}-CA_{CTD} interface, which initiates capsid assembly. Interestingly, the dehydrated native CA-FL structure shows detailed interactions at the 3-fold CA_{CTD}-CA_{CTD}-CA_{CTD} interface that have not been observed in previous crystal structures but have been reported in the cryo-EM-derived all-atom CA model and may play an important role in CA assembly and/or disassembly. Our structure of CA-FL provides a system for the investigation of molecular interactions between CA-FL and small molecule antivirals that work with a novel mechanism of action.

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Recruitment of Superelongation Complexes by Tat

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Superelongation complexes (SECs) are large assemblies organized on a flexible AFF1/AFF4 scaffold protein. SECs are essential for transcription elongation of many human genes, including the integrated HIV-1 genome. At the HIV-1 promoter, the viral Tat protein binds simultaneously to the nascent TAR RNA and the CycT1 subunit of the P-TEFb kinase in a SEC. The kinase phosphorylates negative elongation factors DSIF and NELF, thereby antagonizing their inhibitory action on transcription, and overcoming the promoter proximal pausing of Pol II. HIV viral replication is completely dependent on Tat and the human host cell superelongation complex. To understand the preferential recruitment of SECs by Tat and TAR and their role in HIV transcription, we determined the crystal structure of a quaternary complex containing Tat, P-TEFb, and the CycT binding site of the SEC scaffold AFF4. The structure reveals that the intrinsically disordered proteins Tat and AFF4 fold on the surface of CycT1 and interact directly. Furthermore, the Tat-AFF4 interaction surface is centered on Tat K28, which is acetylated *in vivo* to regulate HIV transcription. Interface mutations in the AFF4 homolog AFF1 reduced Tat-AFF1 affinity *in vivo* as well as Tat-dependent transcription from the HIV promoter. AFF4 binding in the presence of Tat partially orders the CycT1 Tat-TAR recognition motif and increases the affinity of Tat-P-TEFb for TAR 30-fold. These studies suggest that AFF4 contributes to the selective recruitment of SECs by Tat and TAR through a two-stage mechanism, with enhancements of affinity at successive steps.

Architecture of the HIV RNA Export Complex

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Nuclear export of unspliced HIV-1 mRNAs into the cytoplasm is a critical step in the viral life cycle, mediated by the viral accessory protein, Rev. Rev forms an oligomeric complex on the Rev Response Element (RRE), a structured region present in all unspliced and singly spliced HIV mRNAs and enables export by recruiting the nuclear export protein, Crm1, through its nuclear export sequence (NES). The Rev-RRE complex derives its function by building a cooperative assembly using multiple Rev oligomerization and RNA-binding steps, but how these steps are coordinated is unclear. We will describe progress on the crystallography of Rev-RNA complexes that sheds light on the architecture of the Rev oligomer and the role of RNA in complex assembly. To better understand how the RNA helps to scaffold Rev assembly, we utilized SAXS and SHAPE RNA mapping experiments to reveal that the RRE adopts a compact structure stabilized by previously unidentified tertiary contacts. The thermodynamic and kinetic assembly pathways are remarkably similar, consistent with a two-step process in which fast cooperative Rev binding to pre-ordered RRE elements changes long-range contacts between distant RNA sequences and triggers higher-order RNP formation. Our studies reveal sequential Rev binding events on the RRE and support a hierarchical Rev-RRE assembly mechanism. The scaffolding of the RNA and the inherent plasticity and modular nature of the Rev oligomerization surfaces and RNA-binding helices allow the RRE to direct Rev assembly and define the architecture of the Crm1 export complex and possibly complexes with other host proteins during RNA transport and virus assembly.

BACK

EM Structures of Rev Complexes and Beyond

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Single-particle electron microscopy has undergone a revolution in which it is now possible to obtain atomic resolution protein structures and to investigate complexes that may exhibit multiple conformations or dynamic behavior. The HIV Rev protein, which interacts with the Crm1 nuclear export receptor to route viral RNAs to the cytoplasm, forms an oligomeric complex on the Rev Response Element (RRE) RNA that alters its conformation when interacting with different partners. We have used negative-stain single-particle EM to examine the architecture of the Rev-RNA-Crm1 complex and will describe how the oligomeric properties of the proteins organize the assembly of the export complex. While the EM studies of the Rev complexes have so far yielded a low-resolution view, developments in EM technology promise much higher resolution in the near future. In particular, the use of a new generation of direct electron detection cameras and related motion correction technology has provided unprecedented resolution of single particle cryo-EM structures. Furthermore, a Fab assisted single particle cryo-EM approach enables studies of small protein complexes. Such novel cryo-EM technologies are being applied to Rev complexes and other HIV accessory and regulatory complexes currently studied in the HARC Center.

BACK

Electron Tomography of HIV-1 Infection in Gut-Associated Lymphoid Tissue

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Critical aspects of HIV-1 infection occur in mucosal tissues, particularly in the gut, which contains large numbers of HIV-1 target cells that are depleted early in infection. We used electron tomography (ET) to image HIV-1 in gut-associated lymphoid tissue (GALT) of HIV-1–infected humanized mice, the first three-dimensional ultrastructural examination of HIV-1 infection in vivo. Human immune cells were successfully engrafted in the mice, and following infection with HIV-1, human T cells were reduced in GALT. Virions were found by ET at all stages of egress, including budding immature virions and free mature and immature viruses. Immuno-electron microscopy verified the virions were HIV-1 and showed CD4 sequestration in the endoplasmic reticulum of infected cells. Observation of HIV-1 in infected GALT tissue revealed that most HIV-1–infected cells, identified by immunolabeling and/or the presence of budding virions, were localized to intestinal crypts with pools of free virions concentrated in spaces between cells. Fewer infected cells were found in mucosal regions and the lamina propria. The preservation quality of reconstructed tissue volumes allowed details of budding virions, including structures interpreted as host-encoded scission machinery, to be resolved. Although HIV-1 virions released from infected cultured cells have been described as exclusively mature, we found pools of both immature and mature free virions within infected tissue. The pools could be classified as containing either mostly mature or mostly immature particles, and analyses of their proximities to the cell of origin supported a model of semi-synchronous waves of virion release. In addition to HIV-1 transmission by pools of free virus, we found evidence of transmission via virological synapses. Three-dimensional EM imaging of an active infection within tissue revealed important differences between cultured cell and tissue infection models and furthered the ultrastructural understanding of HIV-1 transmission within lymphoid tissue.

BACK

Structure of an ESCRT-III Membrane-Remodeling Complex

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ESCRT-III subunits assemble into membrane-bound filaments in diverse cellular contexts from Archaea to humans. These filaments constrict and sever target membranes, but the structural basis of ESCRT-III mediated membrane remodeling is unknown. Using electron cryo-microscopy (cryoEM), we determined at near atomic resolution how two human ESCRT-III subunits, CHMP1B and IST1, co-polymerize into a double-stranded helical filament. CHMP1B subunits form the inner strand and adopt a highly extended, domain-swapped, and inter-locked conformation; while IST1 subunits form the outer strand and adopt the compact conformation observed in X-ray crystal structures. Using STORM fluorescence microscopy, electron cryo-tomography, and single particle reconstructions, we further document how this filament constricts into polarized cones – an observation that provides insight into the mechanism of membrane remodeling in cellular abscission and other vital membrane remodeling events.

BACK

Activation of the Ubc13-Ub Adduct by TRIM5alpha Requires Dimerization of the RING Domain

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Members of the tripartite motif (TRIM) protein family of RING E3 ubiquitin (Ub) ligases are increasingly recognized for their involvement in regulation of innate immunity. Several TRIM proteins have been shown to promote innate immunity responses by catalyzing synthesis of K63-linked poly-Ub chains. The E3 Ub ligase activity of these TRIM family members is enhanced by either direct or indirect interaction with pathogen-associated molecular patterns (PAMPs), but the mechanism of TRIM involvement in PAMP recognition is not fully understood. In this study we provide further evidence to support the key role of the K63-linkage specific E2 Ub-conjugating enzyme Ubc13 in retroviral restriction by TRIM5α and offer insight into the mechanism of the TRIM5α E3 Ub ligase activity. The crystal structure of the TRIM5α RING:Ubc13 complex reveals that the TRIM5α RING domain forms a dimer in the crystal. Analysis of primary sequence patterns in the TRIM family suggests that RING dimerization is conserved in the majority of human TRIM proteins. Biophysical, biochemical and functional characterization of RING:Ubc13-Ub interactions reveals that RING dimerization is required for activation of the Ubc13-Ub conjugate. The two RING domains within the TRIM dimer are located on the opposite ends of the ~170 angstrom-long rod-shaped molecule, thus the RING dimer is most likely formed as the result of higher-order oligomerization of TRIM5α. Our data elucidate how higher-order oligomerization of TRIM5α contributes to its E3 Ub ligase activity and HIV restriction. The study provides mechanistic insight into the emerging role of TRIM proteins in PAMP recognition.

Cell Biological Mechanisms Underlying HIV-1 Viral Particle Assembly and Release

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The Human Immunodeficiency virus (HIV-1) life cycle involves several highly choreographed steps during which the virus assembles at the plasma membrane (PM) of an infected cell and buds off the membrane as a viral particle. We have used conventional and superresolution imaging approaches to investigate cell biological mechanisms underpinning three key steps in the viral assembly/budding pathway: clustering of the viral coat in the plasma membrane; recruitment of proteins into the viral bud; and virus budding off the membrane. In the first step involving viral Gag coat assembly, we show it is critically dependent on viral and/or host mRNA, which drives Gag clustering through RNA-Gag electrostatic interactions. In the second step involving protein recruitment into the viral bud, we demonstrate that Env proteins incorporate into viral buds through partitioning into a specialized microenvironment created by multimerization of Gag at the PM. In the final step involving viral abscission from the PM, we examine the 3D molecular organization of ESCRT machinery with respect to HIV bud sites to gain critical insight. We find ESCRT-III proteins assemble within the head of the budding virion, not the base as previously proposed. This later finding prompts a reevaluation of current models for ESCRT-III scaffolding, and suggests that ESCRT abscission initiates from within the head of the budding virion.

Specialised Compartments for HIV Assembly – HIV Production in Macrophages

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In HIV infected monocyte-derived macrophages (MDMs), differentiated in culture for more than 7 days, new virus particles assemble in intracellular compartments (IPMCs). These compartments are continuous with the plasma membrane and express many markers that are also found at the cell surface, including the lipid $PI(4,5)P_2$ and the membrane proteins CD44, CD81 and Tetherin. In most MDMs, IPMCs form between 7 and 14 days after monocyte isolation concurrently with the coordinated expression of CD11b,c/CD18 and paxillin, which form focal adhesion-like plaques on the surface of the IPMCs. IPMC integrity requires a functional actin cytoskeleton and integrity is lost following latrunculin treatment or RNAi-mediated knock-down of CD18. Failure to recruit the ESCRT machinery during virus assembly leads to the accumulation of budding intermediates that are mostly confined to IPMCs in IPMC-containing cells.

Tetherin localisation to IPMCs can restrict the release, and the cell-free transmission, of Vpu-negative HIV. In addition, Tetherin restricts the transmission of Vpu-negative virus via direct cell-cell transfer to autologous CD4-positive T cells. Tetherin-mediated restriction is overcome by Vpu expression or RNAi-mediated Tetherin knockdown. However, IPMC formation and maintenance is not dependent on Tetherin expression.

We suggest IPMCs are resident compartments formed in mature MDMs that are used by HIV as the principal site for assembly of progeny virions. *In vivo,* this intracellular site may allow virus particles to assemble in a protected environment, and may allow the virus to exploit macrophages as reservoirs. Moreover, intracellular assembly may facilitate the synchronised release and cell-cell transfer of virus particles through virological synapses.

BACK

Conformational Transition of Membrane-Associated Terminally Acylated HIV-1 Nef and Its Interactions With Host Proteins

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While methods such as X-ray crystallography and NMR are available to determine the structure of folded proteins in solution, few methods are available to resolve structural details for proteins at membranes. Recently neutron reflection (NR) has emerged as a useful method to derive structural information for membrane-associate proteins. We used NR to study the structure of membrane-associated HIV-1 Nef. Nef is one of several HIV-1 accessory proteins and an essential factor in AIDS progression. Nef lacks catalytic activity but instead realizes its functions by interacting with host proteins. Nef exists in both membrane-associated and cytosolic fractions. Membrane association, believed to be essential for all Nef functions, is achieved by an N-terminal myristoylation as well as a cluster of basic residues within the N-terminal arm. NR revealed that upon insertion of the myristate and residues from the N-terminal arm, Nef transitions from a closed to open conformation that positions the core domain 70 Å from the lipid headgroups. This is likely to affect its ability to interact with host proteins by exposing binding motifs on the core domain or by optimally positioning the core domains for interaction with motifs of membrane-associated host proteins. While NR provides the overall residue distribution normal to the membrane, higher resolution information pertaining to specific residues as well as in-plane association are not available from this method. Indeed, dimerization of Nef at the membrane is known to be critical for interaction with some effector proteins. Therefore, we are working to combine NR with hydrogen-deuterium exchange mass spectrometry (HXMS) and with FRET. HXMS has proven very useful in studying protein complexes and the binding of drug molecules to folded proteins in solution. Deuterium exchange of HIV-1 Nef was analyzed in solution and also when membrane-associated. Significant differences in deuterium uptake were detected between the solution form and lipid associated state that will be discussed in comparison to the residue distribution determined by NR for the membrane-associated state. Plans to combine NR and FRET to relate Nef conformation to its oligomeric state and to provide complementary information on the structure of complexes of membrane-bound Nef with host proteins, such as Src family kinases, will also be discussed.

BACK

HIV-1 Env Trimer and Interaction with Broadly Neutralizing Antibodies.

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The discovery and isolation of highly potent, broadly neutralizing antibodies (bnAbs) that recognize a broad diversity of HIV-1 isolates has opened up tremendous opportunities for enhancing our understanding of how HIV-1 can be neutralized. Most of these human monoclonal antibodies are glycan dependent or have to avoid glycans on the Env surface. Structural characterization of a soluble, cleaved version of the Env trimer by X-ray and EM has identified the full extent of antibody interactions on the Env trimer. These bnAbs possess unique features that enable them to penetrate the glycan shield, bind epitopes that consist of glycans and protein segments of Env, and promiscuously adapt to variation in the glycans. Using a combination of structural and biophysical studies, we have identified their mode of binding and analyzed the structural evolution of antibodies within a particular lineage of highly potent glycan-dependent antibodies. Elucidation and characterization of these epitopes, especially in the context of the Env trimer, can provide valuable insights for structure-assisted vaccine design.

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BACK

Program Project on Structure-Based Antagonism of HIV-1 Envelope Function in Cell Entry

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An integrated multidisciplinary approach is employed by this NIH Program Project to identify and mechanistically understand antagonists of HIV-1 cellular entry and infection by targeting the virus envelope glycoprotein (Env) spike. The HIV-1 Env spike functions as a protein machine, with a cascade of protein-protein interactions between the Env gp120 and both the cellular CD4 receptor and co-receptor (usually CCR5) driving conformational rearrangements in the metastable spike down an energy gradient and ultimately enabling membrane fusion at the virus-cell interface. The protein interfaces between gp120 and cellular receptors as revealed by crystallographic structures provide starting targets for structure-based inhibitor investigations in the Program. The overarching philosophy is to identify molecular compounds that could be productive based on mode and breadth of action, to understand how the inhibitors function mechanistically and, ultimately, to define paradigms for prevention and intervention of HIV-1 infection. Molecular design and synthetic progress in the Program have led to a class of small-molecule CD4 mimics (SMCMs) that bind gp120 and in turn function as both full antagonists of cell entry and sensitizers of the virus Env to inactivation by neutralizing antibodies. In addition, a class of peptide triazoles (PTs) has been developed that blocks both gp120 receptor site interactions simultaneously, through conformational entrapment, and causes inactivation and breakdown of virus before cell encounter, thereby opening the way to peptidomimetic antiviral designs. Intriguingly, both SMCMs and PTs have overlapping gp120 footprints that include use of the highly conserved binding site on gp120 for CD4, and so promise the potential for breadth and resistance to escape, yet by different modes of action. Recent screening has also identified a new type of Env gp120 inhibitor typified by the compound 18A; mechanistic understanding of the antagonism is emerging. Ultimately, productive HIV-1 Env antagonists must target the Env trimer. Thus, this Program has incorporated recent technological advancements to investigate the structures, dynamics and inhibitor interactions of membrane-anchored Env trimers. These studies will advance both our understanding of the structure and dynamics built into the virus Env complex and the development of approaches to antagonism based on the intrinsic properties of HIV-1 Env.

BACK

HIV-1 Restriction in Primary T Cells by Stable APOBEC3H Haplotypes

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At least seven distinct APOBEC3H haplotypes exist in the human population. Over-expression studies have indicated that the stability of each enzyme may be related to HIV-1 restriction activity with haplotypes II, V, and VII capable of being expressed stably and restricting Vif-deficient HIV-1 and haplotypes I, III, IV, and VI being weakly or negligibly expressed and failing to inhibit viral infectivity. First, we isolated primary CD4+ T lymphocytes from healthy individuals encoding different *APOBEC3H* haplotypes and used immunoblotting to validate these predictions for endogenous APOBEC3H haplotypes I to V. *APOBEC3H* mRNA levels were similar in donor CD4+ T lymphocytes regardless of haplotype. Second, we found that HIV-1 infection causes *APOBEC3H* transcriptional upregulation and, for stable APOBEC3H haplotypes II and V, this clearly manifests at the protein level. Third, we performed spreading infection experiments using primary CD4+ T lymphocytes to address whether stable and unstable endogenous APOBEC3H haplotypes confer differential susceptibility to HIV-1 infection. Beginning with HIV-1 strain IIIB (lab Vif), we introduced naturally occurring Vif amino acid variations to produce two unique separation-of-function viruses. Similar to lab-Vif, the Vif protein of each virus fully counteracts APOBEC3D, APOBEC3F, and APOBEC3G and enables rapid virus replication kinetics in SupT11 cells expressing these proteins. However, the hyper-Vif protein is better than lab-Vif at counteracting stable APOBEC3H and the hypo-Vif protein is worse, resulting respectively in rapid and restricted replication kinetics in SupT11 cells expressing stable APOBEC3H. Importantly, all three viruses replicated similarly in primary CD4+ T lymphocytes from donors with unstable APOBEC3H haplotypes, whereas only the hyper-Vif virus replicated with robust kinetics in cells from donors with stable APOBEC3H. Lab-Vif and hypo-Vif virus replication was delayed and strongly suppressed, respectively, in the same stable APOBEC3H expressing lymphocytes. These infectivity phenotypes corresponded with levels of APOBEC3H packaging and G-to-A mutation loads in the resulting proviruses. Finally, we noted that the worldwide distribution of predicted hyper- and hypo-Vif alleles coincides with areas of APOBEC3H stability and instability, respectively. Taken together, these data demonstrate that stable endogenous APOBEC3H contributes to HIV-1 restriction and hypermutation, and further suggest that this restriction factor could be a natural barrier to infection by viral isolates with hypo-functional Vif proteins.

BACK

NMR Structure of the HIV-1 Vif Binding Domain of APOBEC3G

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APOBEC3G has a N-terminal Vif binding domain and a C-terminal catalytic domain. Whereas we and others have solved structures of the catalytic domain, structural studies of the N-terminal domain (A3Gntd) have been challenging due to chronic insolubility and inhomogeneity. Therefore, we generated soluble variants of A3Gntd which have >80% sequence identity to the wildtype A3Gntd protein. Some of those soluble A3Gntd variants bound Vif, and were Vif-dependently degraded. We will report the NMR structure of a soluble A3Gntd variant and studies of the interaction between this protein and HIV-1 VIf.

BACK

Structural Studies of APOBEC3s

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We have now solved the crystal structures of three APOBEC3's domains: APOBEC3G, APOBEC3F, and most recently APOBEC3A. In each case, the crystal structure illuminated extensive oligomerization interfaces with intriguing functional implications. We are currently complementing these structures with fluorescent anisotropy binding studies by which we see potent specific dimeric binding of APOBEC3A to its signature DNA binding site. Together these structural and molecular studies suggest novel mechanisms for how oligomerization may mediate substrate recognition and binding.

BACK

Molecular Targets Controlling HIV Latency and Their Exploitation in Cure Strategies

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Despite the remarkable success of intensive antiretroviral drug therapy in blocking the HIV replication, the virus persists in a small number of cells where HIV has been transcriptionally silenced. Interruption of therapy almost invariably results in rapid viral rebound with the consequence that patients need to strictly adhere to life-long treatment regimens.

At the molecular level, viral latency arises when the regulatory feedback mechanism driven by HIV Tat expression is disrupted. Small changes in transcriptional initiation, induced by epigenetic silencing, can lead to restrictions in Tat levels and entry of proviruses into latency. In resting memory T-cells, which carry the bulk of the latent viral pool, multiple molecular mechanisms limiting cellular levels of the essential Tat cofactor P-TEFb and the transcription initiation factors NF-kB and NFAT are combined with epigenetic silencing and recruitment of specific repressors to ensure that the provirus remains silenced unless the host cell is activated. P-TEFb levels are extremely low in resting T-cells due to the absence of the P-TEFb subunit CyclinT1 (CycT1). Immediately following CycT1 induction, P-TEFb becomes sequestered into the nuclear ribonucleoprotein complex 7SK snRNP which serves as an exchangeable pool from which the catalytically competent and transcriptionally active form of P-TEFb can be extracted and directed to genes. Tat efficiently trans-activates HIV transcription elongation by removing P-TEFb from the 7SK snRNP complex and recruiting it to the transcriptionally paused complex at the HIV TAR RNA hairpin. Relatively little is understood how Tat recruits P-TEFb and how 7SK RNP levels are regulated. The recent molecular and structural work on the Tat:P-TEFb complex is the first of many challenging and important structural projects that will inform HIV cure research.

Epigenetic silencing of the latent proviruses, and recruitment of specific repressors is a second area where new molecular targets controlling HIV can be developed. Recent shRNA screens from my laboratory have shown that polycomb repressive complex-2 (PRC-2) and the NURD and SIN3 silencing complexes play critical roles in proviral repression. Unexpectedly we have also found that the Estrogen Receptor-α (ESR-1) was found to act as a nodal regulatory molecule and proviruses can be activated by ESR-1 antagonists and silenced by ESR-1 agonists. Alternatively, activating of key downstream targets in the cellulaer signaling pathways may serve to provide useful signals for proviral reactivation.

In the “shock and kill” strategy to purge the latent proviral pool non-toxic activator molecules (the “shock”) are combined with immunological enhancement strategies (the “kill”). The multiple restrictions imposed on latent proviruses that need to be overcome suggest that proviral reactivation will not be achieved when only a single reactivation step is targeted, but will require both removal of epigenetic blocks and the activation of P-TEFb. Consequently the most successful activating regimens have so far involved using a protein-kinase C agonist to induce P-TEFb and an epigenetic modifier, such as an HDAC inhibitor to substitute for the requirement for transcription initiation factors. Structural studies of the key regulatory complexes will play an important role in exploiting the plethora of potential drug vulnerabilities that could be exploited to selectively induce the provirus in a safe and effective manner.

The Topological Structure of the HIV-1 Rev Response Element RNA

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Nuclear export of unspliced and singly spliced viral mRNA is a critical step in the HIV life cycle. The structural basis by which the virus selects its own mRNA among more abundant host cellular RNAs for export has been a mystery for more than two decades. We applied small angle X-ray scattering to determine an unusual topological structure which the virus uses to recognize its own mRNA. The viral Rev response element (RRE) adopts an "A"-like structure in which the two legs constitute two tracks of binding sites for the viral Rev protein and position the two primary known Rev binding sites ~55 Å apart, matching the distance between the two RNA-binding motifs in the Rev dimer. Both the legs of the “A” and the separation between them are required for optimal RRE function. This structure accounts for the specificity of Rev for the RRE and thus the specific recognition of the viral RNA.

2.9 Å Resolution Crystal Structure of HIV-1 Tat Complexed with Human P-TEFb and AFF4

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University of Nebraska Medical Center

Developing anti-viral therapies targeting HIV-1 transcription has been hampered by the limited structural knowledge of the proteins involved. HIV-1 hijacks the cellular machinery that controls RNA polymerase II elongation through an interaction of HIV-1 Tat with the positive transcription elongation factor P-TEFb which interacts with an AF4 family member (AFF1/2/3/4) in the super elongation complex (SEC). Because inclusion of Tat•P-TEFb into the SEC is critical for HIV transcription, we have determined the crystal structure of the Tat•AFF4•P-TEFb complex containing HIV-1 Tat (residues 1-48), human Cyclin T1 (1-266), human Cdk9 (7-332) and human AFF4 (27-69). Tat binding to AFF4•P-TEFb causes concerted structural changes in AFF4 via a shift of helix H5¢ of Cyclin T1 and the a-310 helix of AFF4. The interaction between Tat and AFF4 provides structural constraints that explain tolerated Tat mutations. Analysis of the Tat-binding surface of AFF4 coupled with modeling of all other AF4 family member suggests that AFF1 and AFF4 would be preferred over AFF2 or AFF3 for interaction with Tat•P-TEFb. The structure establishes that the Tat-TAR recognition motif (TRM) in Cyclin T1 interacts with both Tat and AFF4 leading to the exposure of arginine side chains for binding to TAR RNA. Furthermore, modeling of Tat Lys28 acetylation suggests that the acetyl group would be in a favorable position for H-bond formation with Asn257 of TRM, thereby stabilizing the TRM in Cyclin T1 and provides a structural basis for the modulation of TAR RNA binding by acetylation of Tat Lys28.

Drug Resistance Mutations Alter the Dynamics of HIV-1 Protease

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The development of HIV-1 protease inhibitors (PIs) has been the historic paradigm of rational structure-based drug design, which led to the discovery of potent inhibitors. However, under the selective pressure of therapy, multiple mutations accumulate in protease to confer drug resistance and render PIs ineffective. Characterizing the effect of such mutations on the structure, dynamics, and inhibitor binding energetics of the protease is crucial to the understanding of drug resistance mechanisms and ultimately developing novel and robust inhibitors.

We previously found that a drug-resistant variant, Flap+ (L10I/G48V/I54V/V82A) displays extreme entropy–enthalpy compensation (~10 kcal/mol) relative to wild-type enzyme when binding the highly potent inhibitor darunavir (DRV). In the current study, we address the molecular mechanisms that cause this energetic change and how protease dynamics may be altered due to drug resistance mutations. We investigated and compared the conformational dynamics of wild type and Flap+ HIV-1 protease in unliganded and DRV-bound forms, using extensive molecular dynamics (MD) simulations and NMR relaxation experiments.

Both methods concur in revealing that the conformational dynamic ensemble sampled by the drug resistant enzyme is altered relative to wild type in unliganded and DRV-bound states. These changes may impact conformational entropy and solvation effects in inhibitor binding, possibly due to altered dynamics and dehydration of the flap regions. Our findings highlight the importance of considering entropic contributions and conformational dynamics as an additional challenge in structure-based drug design.

Selection For PI Drug Resistance In Cell Culture: Better Than You Think

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Selection for drug resistance in culture has yielded many insights into resistance pathways yet there is a gap in our knowledge in how to interpret the viral population dynamics in cell culture relative to what occurs in a person. We have used deep sequencing with Primer ID to follow the evolution of resistance to a set of new protease inhibitors as well as to DRV (see Poster by Lee et al.). Given the number of cells in the culture there should be sufficient rounds of replication to place evolution in the deterministic range for single mutations. In addition, there is evidence for massive recombination in the cultures allowing single mutations to be passed around to create combinations of mutations. For these reasons it appears that these cell culture-based selection protocols should avoid evolutionary dead-ends based on stochastic bottlenecks. The selections revealed novel pathways of resistance to sets of inhibitors that differed by single specific side chains providing a robust genetic probe of Protease-inhibitor interaction.

"The role of water in molecular recognition and selectivity"

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Water is an essential part of life and water molecules play a critical role in molecular recognition. We have developed a method based on molecular dynamics and inhomogeneous solvation theory to compute the entropy and enthalpy of hydration sites within the binding site of a protein. The method has been applied to rationalize structure-activity relationships (SAR) for a variety of therapeutic targets, including proteases, kinases, GPCRs, and PPIs. More recently, we have applied the method to study binding selectivity in related proteins and protein variants. Here, we present our recent progress on understanding the role of water in molecular recognition and selectivity, and describe our efforts to apply the method toward HIV protease resistance mutations.

T1. Reactivation of Latent HIV-1 Provirus via Targeting Protein Phosphatase-1

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Eradication of latent HIV-1 provirus requires reactivation of transcriptionally silent proviruses that are not affected by antiretroviral drugs. In latently infected T cells, the lack of transcription factors, such as NF-κB, CDK9/cyclin T1 and viral protein Tat lead to the reduction in viral gene expression. We previously showed that protein phosphatase-1 (PP1) activates HIV-1 transcription by controlling CDK9 phosphorylation. We developed a panel of small molecular compounds targeted to a non-catalytic site of PP1 and found that some of these compounds induce HIV-1 transcription in one round HIV-1 infection and chronically HIV-1 infected T cells and monocytes. We also identified one compound, F3, that activated HIV-1 provirus in HIV-1 Tg mice when applied to the tail of the mice. The F3 compound induced phosphorylation of CDK9 Ser90 residue which we previously showed to upregulate HIV-1 transcription. The F3 compound also decreased CDK9 Ser 175 phosphorylation, which we also showed to regulate CDK9 activity. Molecular dynamics analysis showed that phosphorylation of Ser 175 disrupt CDK9/cyclin T1/Tat complex. Mass spectrometry and immunoblotting analysis of T cells treated with the compound F3 showed increased expression of sds22, a PP1 regulatory subunit. Docking of F3 to PP1 showed that F3 may interact with PP1 via the binding site that accommodates Sds22. Thus, our study identified a novel class of PP1-targeted compounds that activate latent HIV-1 provirus and that may be useful for future anti-HIV-1 therapeutics.

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T2. Purification of 7SK RNP Containing the HIV Tat Co-Factors Cdk9 And CyclinT1

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The HIV-1 Tat protein is essential for viral replication and it acts to activate RNAP II elongation of the integrated provirus. Tat function is mediated by a protein kinase termed P-TEFb. In metabolically active cells, a large portion of P-TEFb is found in a complex termed the 7SK RNP in which the kinase activity of P-TEFb is repressed. The 7SK RNP complex is composed of 7SK snRNA, CDK9, Cyclin T1, HEXIM, LARP7, and MEPCE. Tat can extract CDK9/Cyclin T1 from the 7SK RNP to activate RNAP II elongation of the provirus. Additionally, activation of signaling pathways can release CDK9 and Cyclin T1 from the 7SK RNP, resulting in enhanced viral RNA production. We have developed a purification procedure for the 7SK RNP and our goal is to determine the structure of the 7SK RNP by Cryo Electron Microscopy (CryoEM). We have generated a 293T cell line that expresses a Flag-tagged HEXIM1 protein. A plasmid expression vector for a Streptavidin-tagged Cyclin T1 protein is transfected into this 293T cell line, and the Flag and Streptavidin tags are used for sequential binding and elution of the 7SK RNP. We have analyzed our 7SK preparation by mass spectrometry and found that is composed almost exclusively of CDK9, Cyclin T1, HEXIM1, LARP7, and MEPCE. Thus, our purification strategy appears to be successful and we are currently scaling up our preparation for CryoEM analysis by Dr. Wah Chiu's CRNA CryoEM facility.

T3. Characterization of the HIV-1 Tat – P-TEFb Interaction Using Global Substrate Profiling

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Human immunodeficiency virus (HIV) has a small genome encoding only 15 proteins and is therefore highly dependent on host cellular machinery for replication and proliferation. The physical interactions between each of the viral proteins and various host proteins were recently mapped using an unbiased affinity-purification mass spectrometry-based approach.[1] Ten percent of the 497 high-confidence HIV-host protein-protein interactions identified involve host PTM enzymes, and the activities of these PTM enzymes may be modulated by their interacting HIV factor. Of particular interest is the effect of Tat on the activity of an interacting host kinase, positive transcription elongation factor b (P-TEFb). Tat mediates its transcriptional activation through coordinating P-TEFb phosphorylation of the RNA polymerase II C-terminal domain (RNAP II CTD) and two interacting negative elongation factors. The effect of Tat on the substrate specificity and catalytic efficiency of P-TEFb is unclear, and what serine residue (Ser-2 or Ser-5) within the RNAP II CTD is the preferred substrate of P-TEFb is still unclear.[2] Using our Multiplex Substrate Profiling by Mass Spectrometry (MSP-MS) technology, the effects of Tat and other interacting host transcription factors on the substrate specificity and catalytic efficiency of P-TEFb are being examined.[3] Immunoprecipitated P-TEFb complexes will be profiled using MSP-MS as well in order to describe P-TEFb activity in a more cellular context. Additionally, global phosphoproteomics experiments will be performed in order to further understand the role of Tat and P-TEFb in modulating cellular phosphorylation events.

BACK

T4. Uncovering the Underlying Mechanisms of HIV Transactivation Induced by Post-Translational Modifications in the TAT:AFF4:P-TEFb Semi-Super Elongation Complex

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The HIV Transactivator of Transcription (TAT) is critical to the successful transcription of HIV genes and the HIV genome. Once bound to P-TEFb and other members of the HIV core transcriptional machinery, for example the scaffolding protein AFF4, TAT directs this complex to the HIV Transactivation Response Element (TAR) RNA hairpin still emerging from a transcriptionally arrested RNA Polymerase II (RNAP II). Finally, phosphorylation of both this paused RNAP II and other transcriptional repressors by P-TEFb resumes transcriptional elongation. While it has been well demonstrated that post-translational modifications (PTM's) throughout this complex can either drive an infection towards latency or activation, the molecular underpinnings of these events are largely unclear. Through the use of molecular dynamics simulations of the TAT:AFF4:P-TEFb complex modeled with a range of PTM's, we have explored at the atomic level just how these perturbations are coupled to the biophysical and functional observations seen experimentally. Our approach to studying these PTM's includes i.) using *a priori* knowledge of the system to identify differences across simulations and ii.) utilizing non-biased approaches to uncover less obvious modes of communication between PTM's and both cryptic and established functional sites. Upon preliminary analysis, each system exhibits a unique conformational ensemble in the T-Loop of Cdk9. In particular when compared to the TAT-free complex, the presence of TAT—and to a greater degree the presence of TAT acetyl K28—restricts the conformational freedom of the T-Loop. We hypothesize that constraint of the T-Loop around some active conformer is coupled to HIV transactivation, possibly by increasing Cdk9 kinase activity or stabilizing interactions with the Cdk9 substrates. Importantly, experimental observations characterizing transactivation and kinase activity for these systems corroborate this proposed mechanism. Through these simulations, it is our hope to understand at the molecular level how a miscellany of PTM's regulate the HIV core transcriptional machinery.

T5. Picomolar Ligand for TAR RNA Inhibits HIV Replication in Primary Lymphocytes with Activity Comparable to Current Antivirals

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We reported the discovery of a class of conformationally-constrained cyclic peptides which mimic the HIV-1 transactivator protein Tat and potently inhibit the HIV-1 transactivation response (TAR) RNA in cells. We introduced non-canonical amino acid side chains, based on a structural rationale, to improve the binding activity and achieve greater discrimination against other RNAs. The shorter, non-standard amino acids introduced into the peptide sequence reconditioned the non-optimal contacts, leading to the discovery of a peptide with a binding affinity of 25 pM, a 1,000 fold improvement relative to the previous lead peptide, and unprecedented specificity against even closely related RNAs. The new peptide is as active as current antivirals in inhibiting HIV replication in primary lymphocytes, but achieves inhibition through a new dual mechanism of action. The structure of the new lead compound bound to TAR reveals optimal interactions with the RNA, with the peptide inducing a very deep groove and folding up of the apical loop and bulged nucleotides to make additional interactions with the inhibitor. The improved *in vitro* binding activity and selectivity are unprecedented among other inhibitors and demonstrate that these levels of activity associated with FDA-approved molecules can be obtained for RNA-binding molecules.

BACK

T6. Dynamic-Ensemble Based Targeting of HIV-1 RNA

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The HIV genome contains many regulatory RNA elements that can be targeted in the development of anti-HIV therapeutics. We describe here the application of ensemble-based computational docking in the discovery of small molecules targeting the transactivation response element (TAR) and the exon splicing silencer 3 (ESS3). NMR data is combined with computational molecular dynamics simulations to generate a conformational ensemble of the target RNA. The ensemble of conformations is then subjected to virtual screening using computational docking. Small molecule hits are then interrogated using experimental screening including NMR spectroscopy to map out RNA-ligand interactions. We report the application of this approach alongside conventional high throughput screening using a fluorescence-based binding assay to screen ~100,000 compounds for binding to TAR. With this approach we have identified 11 small molecules with new and favorable scaffolds that inhibit HIV-1 TAR and Tat peptide association with IC50 ranging from 15 mM to 178 mM *in vitro*. One of the compounds exhibits promising activity against HIV-1 single-cycle infection in T-cells and a reporter cell, TZM-bl. We show that the ensemble-based virtual and high throughput screening are complimentary tools that when combined greatly enable the discovery of RNA-targeting small molecules.

T7. RNA-Binding Small Molecules Using Small Molecule Microarrays

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Identifying small molecules that selectively bind to structured RNA motifs remains an important challenge in developing potent and specific therapeutics. Most strategies to find RNA-binding molecules have identified highly charged compounds or aminoglycosides that commonly have modest selectivity. Here we demonstrate a strategy to screen a large unbiased library of druglike small molecules in a microarray format against an RNA target. This approach has enabled the identification of a novel chemotype that selectively targets the HIV transactivation response (TAR) RNA hairpin in a manner not dependent on cationic charge. Thienopyridine 4 binds to and stabilizes the TAR hairpin with a K_d of 2.4 μM. Structure activity relationships demonstrate that this compound achieves activity through hydrophobic and aromatic substituents on a heterocyclic core, rather than cationic groups typically required. Selective 2'-hydroxyl acylation analyzed by primer extension (SHAPE) analysis was performed on a 365-nucleotide sequence derived from the 5' UTR of the HIV-1 genome to determine global structural changes in the presence of the molecule. Importantly, the interaction of compound 4 can be mapped to the TAR hairpin without broadly disrupting any other structured elements of the 5' UTR. Cell-based anti-HIV assays indicated that 4 inhibits HIV-induced cytopathicity in T lymphocytes with an EC_{50} of 28 μM, while cytotoxicity was remarkably not observed, even at concentrations above 1 mM.

T8. A Biophysical Characterization Study of a Potent Small Molecule HIV Inhibitor With Its Target: Didehydro-Cortistatin A/Tat HIV

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The HIV trans-activator of transcription (Tat) protein is mainly involved in HIV transcription. The basic domain (BD) of Tat binds to the mRNA's stem-bulge-loop structure, the Trans-Activation Responsive (TAR) element, located at the 5' terminal region of all HIV mRNAs and recruits the complex p-TEFb to RNAPII activating transcription elongation. Currently, no therapeutic agents are available in the clinic to inhibit Tat. Recently, our laboratory reported that didehydro-Cortistatin A (dCA), an analogue of a natural steroidal alkaloid, 1) inhibits Tat activity; 2) inhibits viral production from integrated viral genomes at the subnanomolar range; 3) prevents proviral reactivation; 4) penetrates in viral sanctuaries unlike current antiretroviral therapy; 5) is not toxic. Here, we biophysically characterize the interaction between active Tat protein and dCA. We produce large quantities of recombinant active Tat protein in E. coli by optimizing codon usage and using nutrient poor medium. dCA efficiently inhibits recombinant active Tat in transactivation based cell assays. dCA specifically binds to Tat in ELISA and Dot Blot assays, and in solution in pull-down assays, as well as bound to an antibody recognizing a 3D epitope on Tat protein. Using peptides encompassing the entire Tat protein in ELISA, we show that dCA binds to Tat's BD and specifically to residues 45 to 55. Moreover, NMR studies with the full-length Tat and peptide of the BD show a specific interaction of dCA with Tat's BD. We also confirm that the association of dCA with Tat prevents Tat binding to TAR, using RNA-EMSA *in vitro.* Blocking the BD of Tat is not only important for HIV transcription but may also benefit other HIV related pathologies. Interestingly, by interacting with Tat, dCA stabilizes the structure of Tat. This is demonstrated by a blue shift of the TRP^{11} fluorescence of Tat, as well as by the ability of dCA to protect Tat from proteolysis in DARTS experiments. The stabilization of Tat folding by dCA gives us a unique opportunity to attempt the crystallization of full-length Tat. The crystal structure of Tat, and more specifically the TAR binding domain of Tat, is crucial for drug development around the Tat target.

T9. Finally: Efficient Quantification of HIV-1 Splicing

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Background: HIV-1 RNA undergoes complex splicing to produce more than 40 different transcripts. Previous research on HIV-1 splicing used artificial constructs and single exon systems, and quantification was done by size and relative abundance of PCR products. It is now possible to quantify HIV-1 RNA splicing patterns in the context of viral infection using Illumina paired end deep sequencing and Primer ID, which prevents quantification errors caused by PCR skewing and oversampling. This quantitative tool can be used to explore the role of splicing regulatory sequences and RNA secondary structures, using splicing patterns as a phenotype.

Methodology: The first step in the assay is to infect cells with HIV-1 or transfect with an infectious molecular clone. Total cellular RNA is extracted and made into cDNA. A key part of this quantification assay is the primers used in this cDNA step. HIV splice variants can be divided into two categories, the 1.8kb and 4kb classes. The 4 kb class includes a large exon which is spliced out of the 1.8 kb class. The 4kb class primer uses a sequence from this exon. The 1.8 kb class primer spans the splice junction. Thus each primer is specific to its size class. Additionally, these two initial primers are tagged with Primer IDs. The Primer ID is a sequence tag used to identify and quantify individual viral RNA templates. Reverse primers are synthesized with a random 10 nucleotide tail sequence. This creates 4^{10} combinations, over a million possible random sequences. Given a sufficient excess of random sequences relative to sample templates, any one viral RNA template will be tagged with a unique random sequence that is then incorporated into the cDNA and all PCR products made from that cDNA. A sorting program interprets the sequencing data to give a pattern of splice junctions. These patterns are first sorted according to Primer ID. All reads with the same Primer ID sequence are condensed into a single splice junction pattern and that pattern is matched to a known splice variant. Regardless off the number of times a Primer ID occurs in the sequencing output, each Primer ID is counted as only one observation of one mRNA structure. This prevents skewing by PCR resampling. The same forward primer is used in the subsequent PCR amplification of both size classes, which also adds Illumina library sequences. Using Illumina paired end deep sequencing it is possible to identify and quantify all HIV splice variants in the context of viral infection

Results: This quantification protocol was used to observe the effect of mutation of SLSA1, a highly conserved stem-loop structure with SA1 (splice acceptor 1) in the loop, on splicing patterns. Two carefully engineered point mutations disrupted the stem without changing the SA1 acceptor sequence or nearby splice regulator sequences and these mutations resulted in an approximately 10-fold decrease in the utilization of SA1. This protocol was also effective to observe and quantify the effect of temperature on splicing patterns and to quantify changes in splicing patterns over time. The assay works in both cells infected by virus or in 293T cells transfected with molecular clones.

Conclusions: The previous attempt to identify the SLSA1 phenotype used the size and abundance of PCR products and, using that technology, misidentified the affected splice site. Given the complexity of splicing patterns it is clear that a quantitative measure based on sequencing of the spliced products will be a much more accurate way to assess changes in splicing patterns.

T10. Undiscovered Cis-Acting RNA Elements in the HIV-1 Genome

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The HIV-1 genome contains a variety of RNA elements that lie within one or more open reading frames. These RNA sequences are multi-functional in that they encode proteins and also perform superimposed functions that are critical during viral replication. Known features in the HIV-1 genome that lie within protein-coding sequences include splice donors, acceptors, and branch points; cis-regulatory elements that enhance or silence nearby splice sites; the rev-responsive element; the central polypurine tract; the central termination sequence; GagPol ribosomal frameshift regulatory elements; and part of the packaging signal. It is not known whether additional essential cis-acting RNA elements exist in the HIV-1 genome.

In an attempt to identify regions of the HIV-1 genome that contain critical but unidentified RNA elements we employed a global silent mutagenesis strategy. A mutant HIV-1 sequence was designed that contained a maximum number of synonymous mutations in all open reading frames while leaving known RNA elements that are important for virus replication intact. To avoid the creation of new splice acceptors and donors, we did not introduce new AG or GT dinucleotides. The large collection of silent mutations was divided into 150-500 nucleotide blocks, which were synthesized and cloned to produce sixteen different mutant proviruses. Despite containing unaltered amino acid sequences, half of the mutants displayed severely impaired fitness during replication in human T-cell lines. Seven of the eight attenuated viruses appeared to be completely incapable of spreading infection but some of these mutant viruses were able to regain fitness through the acquisition of compensatory mutations. The sets of mutations that induced replication defects were spread across the genome and included regions of Gag, Pol, Env, and accessory genes. Three of the defective mutants displayed aberrant splicing, in which some splice products were elevated and others were diminished relative to wild type levels. These observations suggest that the regulation of splicing is influenced by RNA elements that have yet to be identified. Overall, this study demonstrates the largely unexplained genetic fragility that exists in some areas of the genome even when protein sequence is preserved. The large fraction of the HIV-1 genome that is vulnerable to deleterious silent mutations suggests that there are a number of essential non-coding elements that have yet to be discovered.

T11. Structural Characterization of the Intronic Splicing Silencer (ISS) of HIV-1 Using NMR

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Alternative splicing is a crucial process occurring during the human immunodeficiency virus type 1 (HIV-1) life cycle. This process is primarily regulated by interaction between the various splice sites within the viral RNA genome and trans host partners that regulate the splicing process by activating or repressing splice site usage. The host proteins hnRNP A1 and ASF regulate the splicing pattern in HIV-1 transcript by binding to highly conserved 3'splice site ssA7. Splice site A7 is 175 nucleotide long RNA and composed of 3 stem- loop structures that contain splicing regulatory elements ISS (SL1), ESE3 (SL2) and ESS3 (SL3) that bind to trans host proteins and thus regulate the splicing pattern. The mechanism by which this ssA7 recruits these host proteins and regulate the splicing pattern in HIV-1 is not well characterized. The high-resolution solution structure of ssA7 RNA and its interaction studies with its host factors will give the insight about the mechanism involved. Solving the NMR structure of large RNA like ssA7 is challenging and so we have applied divide and conquer approach. The ESS3 solution structure was recently solved and now we are focusing on Intron splicing silencer (ISS).

The ISS is a 50-nucleotide long stem-loop RNA with several non-canonical elements that may add to the dynamics and function of this molecule. Toward understanding the structure-based mechanism of the ISS function, NMR spectroscopy has been used to probe its solution properties. Here, we present a solution structure of ISS.

T12. Elucidation of hnRNP A1 Binding to ssA7 of HIV-1

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Alternative splicing of the HIV-1 genome is necessary for translation of the complete viral proteome. Host proteins, such as hnRNP A1, are used to regulate splicing at the various donor and acceptor sites along the viral genome. One such site regulated by hnRNP A1 is the conserved 3' acceptor splice site A7 (ssA7). Silencing of splicing at this site is necessary in order to retain the Rev Responsive Element (RRE) in the adjacent tat/rev intron. The RRE is responsible for nuclear export of unspliced and partially spliced transcripts.

Our research seeks to clarify the binding determinants of hnRNP A1 on ssA7 by developing a structural model that seeks to correlate ssA7 structure to its splicing function. Initial experiments have used isolated domains of both ssA7 and hnRNP A1. For ssA7, SL3 (ESS3) of the three stem loop ssA7 structure is examined as this contains a high affinity UAG binding site for hnRNP A1. We previously solved the 3D solution structure of SL3 by NMR and found the UAG is located in a terminal heptaloop. Structural studies have been done using the protein UP1, which consists of the two RNA recognition motifs (RRM) of hnRNP A1. Crystallization experiments have yielded a structure of UP1 bound to an AG dinucleotide corresponding to A15 and G16 in the heptaloop of ESS3. This structure shows the two nucleotides are bound in a pocket formed by aromatic residues of RRM1 and the linker between the two RRM domains. NMR experiments with the UP1:ESS3 complex have shown similar results, revealing NOEs between the nucleotides of the terminal heptaloop of ESS3 and the aromatic residues of UP1.

Full length hnRNP A1 has recently been cloned and purified. When the solution structure of ESS3 was solved, the biochemical profile of its binding by the domains of UP1 was analyzed. Identical experiments are being done with full length hnRNP A1 to determine if the presence of the C-terminal glycine rich domain alters the binding of the RNA.

T13. The HIV-1 Rev Response Element Exists in Two Alternative Secondary Structures Which Promote Different Replication Activities

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The HIV-1 Rev Response Element (RRE) is a *cis*-acting RNA element with multiple stem-loops present in all intron-retaining HIV mRNAs. Binding of the Rev protein to the primary binding site, and Rev multimerization on other regions of the RRE, is required for the nucleo-cytoplasmic export of these mRNAs. This is an essential step in HIV replication. However, the precise secondary structure of the HIV-1 RRE remains controversial. Studies have reported that the RRE has either 4 or 5 stem-loops, which differ only in the rearrangement of regions that lie outside of the primary Rev binding site. To understand the role played by these regions, we have examined the relationship between these two structures and Rev-RRE activity.

NL4-3 RRE RNA was folded *in vitro*, analyzed by native PAGE and found to migrate as a "doublet" band. Using in-gel *Selective 2' Hydroxyl Acylation analyzed by Primer Extension* (SHAPE), we found that one of these bands contained an RRE with a 5 stem-loop structure, whereas the other contained a 4 stem-loop structure. Thus, our data demonstrate, for the first time, that the NL4-3 RRE exists in two alternative structures.

To study the significance of these alternative structures, we made RRE mutants predicted to allow only one or the other of the structures to form. The predictions were confirmed using SHAPE. We then compared the activity of the two forms to each other and to the wt RRE. Analysis of the complexes that each RRE formed with purified Rev protein *in vitro* showed differences in their rates of migration on native gels, suggesting structural differences. The RREs were also tested for their abilities to promote viral replication, by inserting each RRE into the Nef region of an RRE-defective provirus which contained RRE mutations that did not change the env protein. Growth kinetics and competition assays showed that the virus with the 5 stem-loop RRE had a higher replicative fitness than the virus with either the wt or the 4 stem-loop RRE. Between the wt and the 4 stem-loop RRE-containing viruses, the virus with the wt RRE appeared more fit. These results suggest that HIV may use two alternative RRE secondary structures to modulate replication, potentially allowing adaptation to environmental demands in time and/or space, analogous to the use of riboswitches in bacteria.

T14. Structural Studies of the HIV-1 and HIV-2 Rev Response Elements

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Structural characterization of HIV-1 and HIV-2 rev response elements (RREs) has been facilitated by the recent development of techniques for probing RNAs that are structurally heterogeneous. Specifically, “ensemble” and “in gel” variations of SHAPE (selective 2’ hydroxyl acylation analyzed by primer extension) allow for extraction of individual structures from a conformeric mixture of RNAs.

Using non-denaturing PAGE and in gel SHAPE, we were able to determine that the HIV-1 RRE is comprised of 5 or 4 stems/stem-loops arranged around a central junction. In terms of secondary structure, these two conformations differ only in the arrangement of nucleotides comprising either stem-loops III and IV or the combined stem-loop III/IV, respectively. High affinity rev binding sites located at the base of stem loop IIB and the purine-rich bulge in stem I appear common to both conformations. A three dimensional model obtained by short angle X-ray scattering (SAXS) indicates that the two high affinity sites are separated by approximately 55 angstroms – the approximate distance separating the nucleic acid binding motifs in *rev* dimers. Based on this observation, it has been suggested that rev oligomerization along the HIV-1 RRE initiates from a rev dimer linking the two high affinity binding sites.

Ensemble SHAPE data indicate that the HIV-2 RRE folds slowly and transitions through two intermediate forms before assuming a final conformation similar to the 5-stem structure reported for the HIV-1 RRE. In contrast to HIV-1, however, the HIV-2 RRE contains a “bridge” of basepaired nucleotides dividing the central junction region into adjacent 4- and 3-way junctions. Moreover, while high affinity *rev* binding sites appear to be present in the HIV-2 RRE, three-dimensional modeling of this element suggests that the motifs housing the two sites are not proximal but are instead arranged coaxially. This roughly linear arrangement of stem loop IIB and stem I is inconsistent with the “bridging” model described above but instead supports the popular “jellyfish” model of rev assembly. A comparison of the two RREs and the different models for rev assembly is provided herein.

BACK

T15. Structural Biology of an HIV-1 Rev-RRE Complex

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The HIV-1 accessory protein Rev mediates the nuclear export of partially spliced and unspliced viral mRNAs for both translation into viral structural proteins and for packaging the viral genome. Rev facilitates this process by oligomerizing on a highly structured region, the Rev Response Element (RRE) present in all partially spliced and unspliced viral mRNAs and by presenting a nuclear export sequence (NES) to recruit the host nuclear export protein, Crm1. Functional Rev-RRE complexes are assembled in a highly cooperative manner through multiple Rev oligomerization and RNA-binding steps, but how these steps are coordinated is unclear. Here, we present our current structural and biochemical investigations on the coupling of RNA recognition and Rev oligomerization and the role of the RRE in assembling the complex, with implications for Crm1 recruitment and nuclear export, and for other potential host protein interactions.

We acknowledge the California HIV/AIDS Research Program postdoctoral fellowship to BJ.

BACK

T16. Assembly of Rev and Cofactor DDX1 on the Rev Response Element

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The HIV-1 Rev (Regulator of Expression of Virion) protein activates nuclear export of unspliced and partially spliced viral mRNAs, which encode the viral genome and the genes encoding viral structural proteins. Rev interacts with a highly conserved region, the Rev Response Element (RRE), located within the viral mRNA. Initially, a single Rev monomer binds to stem-loop IIB of the RRE, whereupon additional Rev monomers are recruited to the RRE through a combination of RNA-protein and protein-protein interactions, resulting in the formation of a functional nuclear export complex. In addition, several cellular host proteins, such as the DEAD box helicase DDX1 are known to be required for efficient Rev function *in vivo*, although their precise role is unknown. In this study, a variety of single-molecule fluorescence spectroscopic methods were used to dissect the role of DDX1 during assembly of Rev-RRE complexes. To facilitate these studies, the large DDX1 protein was enzymatically labeled at the N-terminus with Alexa-647, while Rev was labeled with Alexa-555 at a single cysteine using standard maleimide chemistry. Single-color TIRF measurements with labeled Rev and surface-immobilized full-length RRE (unlabeled) were used to monitor individual Rev monomer binding steps during oligomeric Rev-RRE assembly. Moreover, single-color experiments with labeled DDX1 revealed that this protein also binds directly to the RRE. Two-color colocalization and FRET measurements were used to monitor the simultaneous binding of both Rev and DDX1 to the same immobilized RRE molecule. DDX1 was observed to promote oligomerization of Rev on the RRE, whether or not FRET was observed between Rev and DDX1. When FRET was observed, the FRET efficiency was strongly dependent on the number of Rev monomers bound to the RRE. When a single Rev monomer was bound, the FRET signal fluctuated between three distinct levels, suggesting that DDX1 was exchanging among multiple binding sites on the RRE, each with a different proximity to the bound Rev monomer. In contrast, when two or more Rev monomers were bound to the RRE, the FRET signal was observed within a relatively narrow range, suggesting that one or more of the DDX1 binding sites on the RRE were not available. Together, these studies reveal the complex interplay between Rev and DDX1 binding events during the assembly of the RNP complexes responsible for nuclear export of HIV-1 mRNA. Supported by NIH P50 grant GM082545.

BACK

T17. Roles of DDX1 in Intracellular Trafficking of Unspliced HIV-1 mRNA

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Posttranscriptional regulation of HIV-1 gene expression is critical for virus replication. The HIV-1 transactivation protein, Rev, functions in the late stages of virus replication by promoting the nuclear export and translation of unspliced and partially spliced HIV-1 mRNAs that encode viral structural proteins. Rev binds and oligomerizes at the RRE (Rev responsive element) sequence which is present in the unspliced transcripts, and the Rev/RRE RNP complex is exported by Crm1 pathway. Previously, the DEAD box RNA helicase, DDX1, was identified as a Rev-interacting protein and has been shown to be important for HIV-1 replication and Rev function. DDX1 silencing caused decrease in p24 release from cells and decrease in the cytoplasmic accumulation of unspliced HIV-1 transcripts. However, it remains unclear what is the molecular consequences of the interaction of DDX1 and Rev/RRE complex.

Based on studies of other members of DEAD box helicase family, we propose that DDX1 could influence the composition or structure of the viral mRNP complex, thereby promoting its stability and nuclear export. To test this model, we are studying the physiological impact of DDX1 silencing on the dynamics of Rev/RRE complex. Exo1 cells contain tandem repeats of Tat-inducible HIV-1 transgene with the RRE sequence. This system will allow concerted analysis of Rev-mediated export of unspliced RNA by light microscopy and biochemical assays. We optimized the live-cell imaging system in Exo1 cells and employed FRAP (Fluorescence recovery after bleaching) experiments to assess the dynamics of the nascent transcripts and Rev, respectively, at the HIV transcription site while DDX1 is silenced. This system will be further applied for biochemical studies.

T18. A Mechanistic Role For Human DEAD-Box Helicase 1 (DDX1) in HIV-1 Rev-Dependent Export

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Nuclear export of partially and fully unspliced HIV-1 transcripts is dependent on viral protein Rev binding to, and subsequently oligomerizing on, the HIV RNA Rev Response Element (RRE). DDX1 is a DEAD-box helicase identified as a cellular protein affecting HIV-1 nucleo-cytoplasmic export. However, the mechanism of DDX1 action during export is not clearly understood. Here we demonstrate that the interaction between DDX1 and Rev is specific and of high affinity, being localized to the N-terminus of the conserved DEAD-box domain. Using scanning alanine mutagenesis we have identified several possible residues of Rev required to bind to DDX1 that show overlap with known Rev oligomerization activities. To further understand the role of DDX1 in rev oligomerization we created and characterized several mutants of DDX1 which affect its known enzymatic activities (ATP hydrolysis, ATP binding, RNA binding and helicase). We also present biochemical data indicating a role for DDX1 in HIV-1 RRE StemII rearrangement, independent of Rev binding. This rearrangement is similar to that seen during Rev binding at high concentrations. A model is presented to describe a role and mechanism for DDX1 in nucleo-cytoplasmic export.

BACK

T19. Living Cell Real-Time Single Molecule Imaging of HIV-1 RNA Modulation by DDX1

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Evidence is building that DDX1, a DEAD-box helicase, participates in rev-assisted HIV-1 RNA genome transport processes. We are particularly interested in the spatial and temporal landscape of this rev-RRE and DDX1 interaction in the cell nucleus as well as the binding states. Specifically we aim to understand the life cycle of the HIV-1 genome, beginning at the transcription site of the integrated pro-virus, during intra-nuclear “transport” of the HIV-1 genome (i.e. diffusion to the nuclear periphery) to its appearance in the cytoplasm.

To study HIV-1 RNA and DDX1 interactions in real time in the living cell we engineered an optimized fluorescent cell line which can be used in single molecule microscopy techniques with nanometer and millisecond resolution. This cell line is based on a previously described U2OS derived cell line that harbors 75 stably integrated copies of replication deficient HIV genome labeled using a 24x MS2 cassette (Boireau et al. J Cell Biol. 2007). After selection of a sub-clonal population of cells that express replication deficient HIV genome, we stably integrated a nuclear pore marker (POM121), a MS2 coat protein, a codon optimized DDX1 and shRNA(s) against endogenous DDX1. All integrated sequences are fused to a fluorescent protein sequence except for the shRNA(s). Cell populations were selected either by fluorescence levels or via antibiotics in case of the shRNA(s). We show that the codon optimized DDX1 can rescue a full depletion of endogenous DDX1. This allows us to modify the amount of labeled, optimized DDX1 relative to endogenous DDX1 and further optimize our cell line for all three fluorescent tags to visualize and track HIV-1 RNA and DDX1 molecules in real-time in living cells by single molecule microscopy.

T20. The Role of HIV-1 Genomic RNA Secondary Structure on Frameshifting *In Vivo.*

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The expression of the human immunodeficiency virus type 1 (HIV-1) *gag-pol* polyprotein occurs via a ribosomal frameshift between the *gag* and *pol* genes. The frameshift efficiency is approximately 5% and determines the *gag* to *gag-pol* polyprotein ratio, which is important for viral replication and infectivity. The HIV frameshift site has therefore been hypothesized to be an attractive therapeutic target. The frameshift site consists of a heptanucleotide slippery sequence (UUUUUUA) followed by a downstream RNA stem-loop structure. We have previously shown that *in vitro*, there is a strong correlation between frameshift efficiency and the thermodynamic stability of the first 3 base pairs in the stem-loop (Mouzakis et al., Nucleic Acids Res. 2013, 41(3):1901-13). Thus, the frameshift mechanism must involve a thermodynamic block to ribosome translocation. However, we also observed that frameshift efficiency is modulated by the surrounding genomic secondary structure, perhaps by controlling the polyribosome density at the frameshift site.

We have now investigated the role of HIV-1 secondary structure on frameshifting *in vivo*. Using 293T cells transfected with different mutant provirus plasmids, we show that the frameshift efficiency is thermodynamically controlled in a manner that is striking similar to our previous *in vitro* results. Additionally, we have further dissected the role of genomic secondary structure on frameshift efficiency. Viruses resistant to protease inhibitors frequently acquire a C to U mutation downstream of the frameshift site. We demonstrate that this mutation creates a secondary frameshift site that allows increased production of gag-pol, even when the primary frameshift site is disrupted. We further show that the secondary frameshift site is dependent upon the surrounding genomic secondary structure.

BACK

T21. Chemo-Enzymatic Labeling Methods to Decode Encoded RNA Messages

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In response to various cellular cues, RNAs can modulate the expression of various genes by recoding chemical signals that form an integral part of their 3D architecture. While X-ray crystallographic studies have provided significant insights into how RNAs recognize their cognate ligands, the nature of the unbound state remains elusive for most RNAs that require switching their structure for function. Here we outline strategies for illuminating the nature of these poorly characterized states. These include new chemo-enzymatic synthetic methods and NMR experiments to accurately characterize RNA structural dynamics. Emerging themes of ON and OFF signaling afforded by these genetic switch elements would be highlighted, and applications of these strategies to problems in HIV biology would also be presented.

BACK

T22. NMR Spectroscopy of Large RNAs Using *In Vivo* Labeled RNAs

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In the last two decades, RNA has emerged as more than a messenger molecule. It is now clear that RNA has numerous functions, including enzymatic activity, regulation of gene expression, and control of viral infectivity. Such critical gene expression are dependent on RNA structures undergoing folded and unfolded transitions. The exploitation or modulation of such "switching" activities of RNAs as potential therapeutic targets requires a detailed understanding of their three-dimensional architecture and dynamics.

Nuclear Magnetic Resonance (NMR) spectroscopy is an attractive method of studying RNA structure and dynamics in solution, without worrying about crystallization. However, two disadvantages of NMR must be overcome, especially when studying molecules of large molecular weight: spectral overlap of resonances and broadened linewidths. Furthermore, common methods of RNA purification involve denaturation and renaturation of the RNA, leading to the presence of artificially folded RNAs in the sample.

The resolutions of NMR spectra can be significantly improved by the site-specific labeling of RNA with NMR-active isotopes, ^{15}N and ^{13}C. Using genetically engineered *E. coli* mutants, we have developed a method for large-scale, *in vivo* production of site-specifically labeled RNA. To prevent the RNA from being degraded by nucleases in the cell before it can be harvested, our construct includes a tRNA scaffold that disguises the RNA of interest. Our purification protocol excludes commonly-used denaturation steps, allowing the RNA to maintain its co-transcriptional fold. We will showcase our efforts to streamline *in vivo* labeling for selected RNAs. Our long-term goal is to determine how these RNAs change from an unfolded structure to one with the ability to recognize their cognate ligands.

BACK

T23. Recent Advances in NMRViewJ for Analysis of RNA

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NMRViewJ is one of the most widely used programs for the visualization and analysis of macromolecular NMR spectra. Development of specialized features in NMRViewJ has focused primarily on its application to spectra of proteins. We describe here recent work intended to develop NMRViewJ into a powerful tool for analysis of the NMR spectra of RNA. In order to facilitate the assignment and validation of RNA chemical shifts we developed a predictive model that uses as inputs the primary sequence and secondary structure attributes (helices, loops, bulges etc.). The model was trained using both proton and carbon chemical shifts deposited at the BMRB and performs very well. The model was then integrated into NMRViewJ so that the user can enter an RNA sequence and secondary structure and immediately generate predicted values for the chemical shifts. Based on these predicted shifts, and experiment-specific connectivities, we can then generate a simulated peak list with a network of connections. An interactive tool allows the user, while observing the matching of a whole network of peaks, to adjust the simulated peak list to coincide with measure peak positions. We also describe our ongoing work to incorporate structural information in the RNA analysis. The chemical shift prediction tool is being extended to incorporate 3D structural information, including ring-current shifts. Structure calculation methods based on modern optimization software and coarse grain RNA models are being incorporated to provide structural feedback early in a project. In addition, we describe ongoing work aimed at improving the overall analysis workflow by integrating data processing. A new data processing engine that automatically uses multi-core processors and non-uniform sampling methods is under development.

T24. RNA Secondary Structure of HIV-1 Gag Using Phylodynamics and Covariation Analysis

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Background: The RNA secondary structure of the HIV-1 genome, including *gag* p24, was recently defined using SHAPE analysis (Watts et al. 2007). Disease progression in HIV-1 infected patients is associated with the presence or absence of the p24-specific HLA B*57 allele. Phylodynamic data for HIV-1 *gag* in patients who lack HLA B*57 indicates less constrained evolution than for patients with HLA B*57. By mapping this information on the proposed SHAPE secondary structure for *gag* p24, it is possible to identify sites where covariation helps preserve paired regions in the secondary structure. This approach, together with the SHAPE data, helps to refine the overall secondary structure for this region and suggests that pairing phylodynamic analysis with evolving structural data could help resolve RNA structures throughout the entire HIV-1 genome.

Methodology: Longitudinal HIV-1 p24 sequences were sampled from 6 HIV-1-infected patients characterized by absence of the p24-specific B*57 HLA allele. These sequences were aligned and a consensus sequence was generated that represented all patients. The consensus sequence was threaded onto the proposed SHAPE structure, and then all inter-patient changes were mapped by position, generating maps for each patient over time and a single secondary structure map representing all non-B*57 patients. By overlapping the highest quality SHAPE information (for both paired and unpaired regions), together with the phylodynamic data, and further validating likely stems with mfold, we have arrived at a revised secondary structure for *gag*.

Results: Phylodynamic data for this population indicates a series of rapid adaptive periods within each patient, followed by periods of stasis. There is a broad range of changes from positions that are infrequently modified to those that are changed often. Mapping these more frequent changes on the proposed SHAPE structure helps define nucleotide substitutions that preserve paired regions through covariation. While SHAPE had earlier predicted that many of these positions would be paired, other regions predicted to be unpaired by SHAPE are likely to be paired in light of the phylodynamic information.

Conclusions: The approach outlined in this paper supports the use of phylodynamic information to assist in the development of secondary structure maps. The use of SHAPE, phylodynamics, and computational-structure-prediction algorithms provides a better-defined structure than any one approach alone, with positions supported by multiple lines of evidence. This same approach could be applied to any portion of the HIV-1 genome where phylodynamic information is being generated, however this requires sampling of sufficient sequences to derive useful information about covariation at specific sites. We will repeat this analysis using phylodynamic data for patients with the p24 B*57 allele to determine to what degree the gag secondary structure between these patients is associated with the effects of HLA B*57.

BACK

T25. Electron Cryo-Microscopy of Retroviral RNA

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Electron cryo-microscopy (cryo-EM) is a powerful structural technique that can now routinely reach better than 4 Å resolution for large, homogeneous specimens, such as protein machines and viruses. The advantage of cryo-EM is that prior to imaging, specimens are frozen instantaneously in liquid ethane, which allows them to retain a near native hydrated state. Recently, we began exploring whether cryo-EM, combined with novel developments in the field, is a viable technique to study small, heterogeneous nucleic acids. Our approach includes utilizing both the latest optics developments in the field (Zernike Phase Contrast Optics) as well as experimenting with various data acquisition schemes for optimal contrast and resolution of these small molecules. This methodology has been applied to retroviral RNAs of sizes between 31 kDa and 110 kDa, some of the smallest molecules to be studied by cryo-EM. In addition, we often use a combination of single particle cryo-EM and tomography (cryo-ET) to resolve the structure of these RNAs. Our focus currently lies not only in optimizing data acquisition but also developing and improving the computational methods applied during data processing.

BACK

T26. Selective Pressure at the Level of Structured RNA for HIV-1 p24: The Need for Quantitative Selection Measures

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Background: The HIV-1 gag p24 capsid protein is conserved throughout evolution of the virus through purifying selection of synonymous amino acid substitutions. Studies of the HLA-B*57 allele, associated with slower disease progression, indicated that p24 diversity in patients with HLA-B*57 was more constrained when compared to patients without the HLA-B*57 allele. Of interest is how this altered diversity may relate to RNA structure. The extent of purifying selection, either for the protein as a whole or for individual sites, is determined by calculating the ratio of synonymous to otherwise non-synonymous substitutions. However, current selection analysis methods do not account for selection at the level of RNA, the secondary and even tertiary structures of which are known to influence the replicative life cycle of the virus. Therefore, for this study, nucleotide substitutions were analyzed for covariation and mapped onto a portion of the predicted p24 RNA secondary structure (Watts et al. 2007) in order to determine the extent of RNA base pair alterations among nucleotide changes that do not confer changes at the amino acid level.
Methodology: Longitudinal HIV-1 p24 sequences were sampled from 12 HIV-1-infected patients characterized by the presence or absence of the p24-specific B*57 HLA allele. Nucleotide substitutions persisting for more than 1 sampling time point were categorized by their effect on the corresponding amino acid (synonymous or non-synonymous) and RNA intra-helical base pairing (paired or unpaired). Compensatory substitutions were determined for individual regions of approximately 50 nucleotides using the pairwise covariation method implemented in CoMap.
Results: The ratio of synonymous to non-synonymous substitutions was more than twice as great for B*57 patients than for non-B*57 patients. Interestingly, this result was primarily due to the 2.5-fold greater number of synonymous substitutions. Alternatively, based on the pairing reported in the Watts, et al. (2007) structure, less than 50% of synonymous substitutions maintained helical base pairing in both cohorts. No shared compensatory substitutions were observed for patients within either group.
Conclusions: The high ratio of synonymous to non-synonymous substitutions in viral sequences sampled from patients with the HLA B*57 allele indicated that p24 in these patients had experienced purifying selection, a likely result of reduced fitness for variants expressing escape mutations. At the RNA level, however, the majority of these substitutions disrupted base pairings in the helical regions determined by Watts, et al. (2007), which were not accompanied by compensatory substitutions. Further work to determine the effect of these changes on viral function and fitness is required. However, with the expanding knowledge of functional roles for RNA structure in protein coding regions, purifying and diversifying selection at the RNA level should also be considered when studying evolutionary patterns that may contribute to the fitness and replicative ability of the virus.

T27. Characterization of the Monomeric Conformation of the HIV-1 RNA 5'-Untranslated Region

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The three-dimensional (3D) structure of the monomeric conformation of the 5'-untranslated region (5'-UTR) of the HIV-1 genomic RNA is not known. The 5'-UTR is the most highly conserved region of the HIV-1 genome and controls a number of important steps in the viral life cycle. Research suggests that the 5'-UTR has two primary 3D conformations: monomeric and dimeric. The 5'-UTR acts a structural switch in the viral life cycle with the monomeric conformation of the 5'-UTR promoting translation of the genome, while the dimeric conformation promotes packaging of the genome into new viral particles. Solving the 3D structures of both the monomeric and dimeric conformations will increase our understanding of the mechanism of RNA dimerization and packaging in HIV-1, aiding the development of new therapeutics. The 3D structure of the core dimeric conformation is being investigated via nuclear magnetic resonance (NMR) analysis by the Summers lab. Recent NMR studies utilizing a novel NMR probing technique, long-range Adenosine Interaction Detection, elucidated the base pair interactions stabilizing the monomeric conformation. This allowed the creation of a monomeric, translation-enhanced, 5'-UTR construct for structural studies. Comparison of 2D NOESY spectra of the monomeric 5'-UTR to spectra of predicted elements in the 5'-UTR have allowed portions of the complete monomeric 5'-UTR to be assigned. The TAR hairpin, DIS hairpin, Splice-Donor hairpin, and Ψ-hairpin appear in the monomeric 5'-UTR. Further structural studies will elucidate the complete structure of the monomeric conformation of the 5'-UTR.

BACK

T28. Structure of the HIV-1 Core Encapsidation Signal.

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During assembly of the human immunodeficiency virus type 1 (HIV-1), two copies of the unspliced viral RNA genome are efficiently incorporated into nascent virus particles. Dimeric genomes are selected for packaging through interactions between the nucleocapsid (NC) domains of Gag and the packaging signal, located within the 5'-leader (5'-L). Previous studies identified a minimal region of the HIV-1 5'-L which is required for packaging, a Core Encapsidation Signal, 5'-L$^{344\text{-}\Delta TAR\text{-}\Delta Poly(A)\text{-}\Delta PBS}$. Using ^{2}H-edited Nuclear Magnetic Resonance spectroscopy, and a fragmentation-based approach, we have determined the structure of the Core Encapsidation Signal, Ψ^{CESm}, in which the palindromic loop of the dimerization initiation site (DIS) has been mutated to prevent RNA dimerization. This RNA adopts an unexpected tandem three-way junction structure, in which the major splice donor site and the *gag* start codon are sequestered, while a number of guanosines, which may serve as NC binding sites, are exposed. The structure of Ψ^{CESm}, a packaging-competent RNA, explains not only the increased number of high-affinity NC binding sites, but also the attenuation of splicing and translation.

BACK

T29. Distinctions in HIV-1 RNA Structure and Host Components Between the Translation RNP and the Packaging RNP

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HIV-1 encodes a single, multifunctional transcript that serves as mRNA template for synthesis of viral structural proteins and as virion RNA template for reverse transcription. Overlapping within the 5' untranslated region (UTR) are molecular determinants of ribonucleoprotein particles (RNPs) that carry-out the processes of translation, encapsidation, or balanced splicing of the unspliced transcript. A major focus of the CRNA is to define the distinct form and function of the HIV-1 RNPs because they are fundamental aspects of HIV-1 infection and persistence.

RNA affinity/proteomic analysis of HIV-1 5'UTR and validation studies identified host protein components of the translation RNP and the packaging RNP. Co-immunopreciptiation and domain analysis demonstrated N-terminal residues (N-term) of DHX9/RHA specifically and selectively recognize structural features of the 5' RNA terminus corresponding to RU5 and PBS [TAR-poly(A)]. The specific recognition of TAR-pol(A) tethers ATP-dependent activity that stimulates cap-dependent ribosome scanning and polyribosome accumulation on the gag-pol open reading frame.

Semiquantative analysis determined that RHA is incorporated into HIV-1 in stoichiometry similar to the virion RNA, ~2 on average. Virions made from RHA-deficient cells exhibit normal genome RNA content, but diminished reverse transcription activity. RHA's incorporation requires its N-term RNA binding domain and HIV-1 leader nucleotides that overlap the packaging signal (psi). While ATPase activity was necessary for HIV-1 mRNA translation, nonenzymatic chaperone activity was sufficient for RHA incorporation and rescue of viral infectivity; thus implicating a distinct molecular basis for activity of RHA at TAR-poly(A) or distal gag mRNA leader. CRNA biophysical studies had defined structural conformers of the 5' UTR that mimic thermodynamic equilibrium between AUG-exposed and AUG-constrained 5' UTR. These were introduced into HIV-1 provirus and studied in transfected cells. The efficiency of gag translation was significantly different between the isoforms and validated the projection of distinct structural conformation of the HIV-1 translation RNP. Our results are important progress in the CRNA's investigation defining fundamental tenets of HIV-1 RNA biology and therapeutic options.

BACK

T30. Global Identification of the RNA Targets of HIV-1 Gag During Virion Genesis

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HIV-1 Gag protein selectively packages two copies of the viral genome, an event thought to be primarily mediated by interactions between the packaging signal (Ψ) on the viral genome and the nucleocapsid domain of Gag. However, the Ψ sequence is defined based primarily on genetic studies and limited in vitro binding data. To date, no assay has been able to demonstrate a direct and specific interaction between Ψ and Gag protein in a relevant context, i.e. in live cells and in virions. Importantly, deletion of Ψ does not completely abolish genome encapsidation, suggesting that packaging may be more complex than Ψ:Gag interaction, and other regions on the viral genome may contribute. In addition, Gag undergoes several changes in localization, multimerization state and is proteolytically processed during particle genesis, but it is completely unknown how these changes affect RNA-binding properties of Gag. Finally, based on in vitro studies, the matrix (MA) domain of Gag has also been suggested to bind viral and/or cellular RNAs, yet the relevance of this finding has never been tested in a cell-based system. Therefore, we set out to determine globally and at near-nucleotide resolution the RNA targets of Gag during various stages of virion genesis, in cells and in virus particles.

To identify the RNA targets of Gag, we adapted a CLIP-seq (crosslinking-immunoprecipitation-sequencing) methodology, which combines immunoprecipitation of covalently crosslinked protein-RNA complexes with high-throughput sequencing. This method globally identifies the RNA molecules associated with an RNA-binding protein of interest in biological settings and provides near-nucleotide-resolution information about the protein-RNA interaction sites. In addition to determining precisely where within Ψ Gag is bound, we identified several novel sites on the viral genome that are specifically bound by Gag in the cytosol of cells. Mutation of these sites delayed virus replication and we are currently investigating whether this is due to defects in genome packaging. Experiments performed on fractionated cells, mature and immature virions indicated that there are major changes in RNA binding specificity during particle morphogenesis. Surprisingly, even though the MA domain of Gag did not bind viral RNA, a few distinct classes of tRNAs were specifically bound by MA in the cytosol, which appears to play a role in regulating the association of MA with the plasma membrane. Finally, we have also globally identified several cellular RNA molecules, such as 7SL RNA, that are specifically bound by Gag in the cytosol and encapsidated into virus particles.

Our studies indicate that Gag selects viral genome for packaging by binding to a few distinct regions on the viral genome and that association with the plasma membrane and particle formation induces significant changes in the RNA-binding properties of Gag. These studies provide the first dynamic, quantitative and high-resolution picture of viral/cellular RNA interactions with Gag in relevant biological contexts.

BACK

T31. Determining the Role of Nuclear Trafficking of the Retroviral Gag Protein

Rebecca J. Kaddis[1], Breanna L. Rice[1], Estelle Chiari-Fort[1], Timothy Lochmann[1], Matthew Stake[1], Nikoloz Shkriabai[3], Mamuka Kvaratskhelia[3] and Leslie J. Parent[1, 2]

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The retroviral Gag polyprotein selects genomic RNA for encapsidation through a high-affinity interaction with the psi packaging sequence in the 5' leader sequence. However, the mechanism by which Gag finds the genomic RNA amidst the sea of cellular RNAs in the infected cell is not well understood. It was originally thought that Gag functioned entirely within the cytoplasm. However, recent evidence suggests that the Gag proteins of multiple retroviruses, including feline immunodeficiency virus (FIV), human immunodeficiency virus (HIV), murine leukemia virus (MLV), mouse mammary tumor virus (MMTV), and Rous sarcoma virus (RSV), traffic through the nucleus.

Studies from our laboratory have provided evidence for nuclear and nucleolar trafficking of HIV Gag and NC using confocal microscopy. Similarly, the Gag protein of MMTV also localizes to nucleoli. For most retroviruses, the role of Gag nuclear trafficking remains unclear.

For RSV, our data suggests that a linkage exists between Gag nuclear trafficking and genomic RNA packaging. Confocal microscopy revealed that Gag may be associating with nascent viral and non-viral RNAs at sites of active transcription. Similarly, affinity purifications and cellular fractionation experiments revealed that Gag may be interacting with chromatin-remodeling proteins, transcription-associated proteins, and splicing factors in association with heterochromatin and euchromatin fractions. Together, these data support a model of co-transcriptional packaging of RSV RNA by Gag. Gag is tethered to a nuclear host factor, perhaps in association with chromatin, near sites of active transcription, the highest local concentration of unspliced viral RNA. Gag then "samples" sites of active transcription by virtue of the non-specific nucleic acid binding ability of the NC domain. When Gag finds the viral RNA, it binds to psi to form a viral ribonucleoprotein complex, that is exported from the nucleus by Crm1:RanGTP, and trafficked to the plasma membrane for assembly.

BACK

T32. Membrane Coupling of HIV-1 MA Domain to Lipid Bilayers: Factors that Influence Affinity and Protein Layer Structure

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The structural protein Gag is an essential component in a number of retroviral pathogens for the assembly of daughter virus particles within infected host cells. In HIV-1, Gag targets the plasma membrane specifically through its N-terminal matrix (MA) domain which is myristoylated in HIV-1. Proper assembly and viral particle budding then requires a subsequent reorganization of the Gag structure at the membrane surface. With quantitative binding assays and neutron scattering from carefully optimized planar bilayer samples, we study the energetics and structure of Gag assembly and its reorganization on artificial membranes that mimic the plasma membrane in their composition.

To disentangle the electrostatic, hydrophobic and lipid-specific interactions of MA with the bilayer surface, their individual contributions were measured using surface plasmon resonance (SPR). Those measurements are straightforward for unmyristoylated MA and revealed a moderate affinity due to electrostatic attraction between the protein and the membrane. In distinction, myristoylated MA showed unexpected results which suggest that myr-MA forms more than a monomolecular layer of protein at the membrane surface. In addition, SPR titration indicated that high myr-MA solution concentrations promote a slow aggregation process on the bilayer surface. Neutron reflectivity corroborated the SPR results and showed material density beyond a primary layer of MA protein bound to the membrane surface.

Here, we investigate these findings, interpreted as a propensity of myr-MA to aggregate either already in solution or on the bilayer surface, as a function of membrane composition and buffer composition. Using electrospray ionization mass spectrometry, we characterized the chemical composition and confirmed the purity of myr-MA from various sources, *i.e.*, different laboratories that purify myr-MA with distinct protocols. Dynamic light scattering and size exclusion chromatography showed a tendency of MA to form oligomers in solution. Purification steps that removed these oligomers resulted in SPR response consistent with the formation of homogeneous protein monolayers at the membrane surface.

Preliminary SPR measurements using improved preparation protocols for myr-MA indicate that myristoylation increases protein affinity to membranes with 30% PS (the typical concentration of charged lipids in the inner plasma membrane) by >10-fold compared to unmyristoylated MA that is attracted to the bilayer surface purely by electrostatic interactions. 20% cholesterol in these bilayers increases protein binding further, but does so only moderately. However, a low percentage of phosphatidylinositol-4,5-bisphosphate, $PI(4,5)P_2$, boosts membrane affinity of myr-MA by another order of magnitude. NR measurements are currently underway to determine the MA layer morphology under these conditions.

BACK

T33. Structural Basis for Membrane Targeting by the HIV-1 Matrix Protein

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The N-terminal matrix (MA) domain of HIV-1 Gag mediates membrane targeting and binding through exposure of an N-terminal myristate moiety and interactions with cellular phosphatidylinositol-4,5-bisphosphate [PI(4,5)P_2] molecules. These events are believed to permit Gag tangential movement and localization to lipid rafts. Despite strong evidence on the importance of both PI(4,5)P_2 and the myristate, the mechanism by which PI(4,5)P_2 interacts with MA in the context of a membrane and how Gag moves to lipid rafts has yet to be established. Here we have employed model membranes, bicelles and liposomes, displaying physiological molar percentages of native PI(4,5)P_2 to investigate how it interacts with HIV-1 MA. Using NMR, our results show that native PI(4,5)P_2 binds to conserved residues on MA, mediating membrane selection.

BACK

T34. Novel Techniques to Study Gag-Membrane Interactions In Vitro

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The interaction of the retroviral Gag protein with the inner leaflet of the plasma membrane (PM) is governed by several factors, including negative membrane charge due to phosphatidylserine (PS) and phosphatidylinositol phosphates (PIPs). We previously demonstrated that the addition of cholesterol to liposomes, thus increasing lipid order, augments Gag-liposome binding. On the other hand, the replacement of lipids carrying saturated acyl chains with lipids carrying unsaturated acyl chains, thus decreasing membrane order, also augments Gag-membrane binding. The underlying mechanisms to explain these results, which were obtained with the classic but cumbersome liposome flotation, remain to be clarified. We are exploring several novel methods to study membrane binding more efficiently and in a more biologically relevant manner. One uses fluorescence correlation spectroscopy (FCS) to score the fraction of a fluorescently labeled protein that is bound to liposomes and thus diffusing slowly. In principle this technique could speed up binding analyses by a factor of a 100. Another technology uses neutron scattering to probe the details of protein-membrane interaction, for example to determine the extent to which a protein penetrates the hydrophilic lipid head group region of a bilayer. In a third method, we are preparing asymmetric bilayers both in large unilamellar vesicles (LUVs) and giant unilamellar vesicles (GUVs). To date almost all protein-membrane studies in vitro have been carried out with symmetric bilayers, which therefore do not mimic the PM. Preliminary data from these studies will be presented.

BACK

T35. Biophysical Studies of RSV Gag Conformation and Membrane Binding.

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For most retroviruses, association of the retroviral Gag protein with the inner leaflet of the plasma membrane (PM) is a critical step in the production of enveloped virus particles. The N-terminal MA domain of Gag binds to the PM using a combination of interactions, including electrostatic, hydrophobic (due to myristoylation), and binding to phospholipid head groups such as that of PI(4,5)P2. Using a non-myristoylated form of purified HIV-1 Gag and the techniques of size exclusion chromatography (SEC) and small angle X-ray scattering (SAXS), it was demonstrated previously that HIV-1 Gag is flexible and compact in solution, with the MA and NC domains close together. Additionally, low angle neutron reflectometry (LANR) of HIV-1 Gag bound to a supported lipid bilayer suggested that both the MA and the highly basic NC domain can simultaneously interact with the bilayer. In contrast to HIV-1 Gag, non-myristoylated MLV Gag was shown to be rod-like in solution. Nevertheless, by LANR both the MA and NC domains of MLV Gag were inferred also to be able to simultaneously interact with a membrane. Interpretations of these results for HIV-1 and MLV are complicated by the fact that in vivo myristoylation is essential for PM binding. By contrast, RSV Gag is not naturally myristoylated, and it can be purified in concentrated soluble form, making biochemical observations more relevant to the biology of the virus. By analyzing highly purified RSV Gag by SEC and SAXS, we show that RSV Gag prefers a rod-like conformation. Preliminary analysis by LANR of RSV Gag bound to a supported bilayer suggests that like HIV-1 and MLV Gag, both the N- and C-terminal domains of Gag can bind simultaneously to the membrane. Taken together these results suggest that while the solution conformation of Gag proteins may vary, the proteins share the ability to interact with membrane bilayers with both ends. We are probing the mechanisms underlying dual domain membrane interaction in the tractable and biologically relevant RSV system.

BACK

T36. HIV-1 Tat Membrane Interaction Probed Using X-Ray and Neutron Scattering, CD Spectroscopy and MD Simulations

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We report the effect on lipid bilayers of the Tat peptide $G_{48}RKKRRQRRRPPQ_{60}$ from the HIV-1 virus transactivator of translation (Tat) protein. Synergistic use of low-angle X-ray scattering (LAXS) and atomistic molecular dynamics simulations (MD) indicate Tat peptide binding to neutral dioleoylphosphocholine (DOPC) lipid headgroups. This binding induced the local lipid phosphate groups to move 3 Å closer to the center of the bilayer. Many of the positively charged guanidinium components of the arginines were as close to the center of the bilayer as the locally thinned lipid phosphate groups. LAXS data for DOPC, DOPC/dioleoylphosphoethanolamine (DOPE), DOPC/dioleoylphosphoserine (DOPS), and a mimic of the nuclear membrane gave similar results. Generally, the Tat peptide decreased the bilayer bending modulus K_C and increased the area/lipid. Further indications that Tat softens a membrane, thereby facilitating translocation, were provided by wide-angle X-ray scattering (WAXS) and neutron scattering. CD spectroscopy was also applied to further characterize Tat/membrane interactions. Although a mechanism for translation remains obscure, this study suggests that the peptide/lipid interaction makes the Tat peptide poised to translocate from the headgroup region.

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BACK

T37. HIV-1 Envelope Glycoprotein Incorporation Requires Matrix Trimerization

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The matrix (MA) domain of HIV-1 Gag plays key roles in Gag targeting to the plasma membrane and envelope (Env) glycoprotein incorporation into virions. Although a trimeric MA structure has been available since 1996, evidence for functional MA trimers in either cells or virions has been elusive. The mechanism by which HIV-1 Env glycoproteins are recruited into virions likewise remains unclear. We can now show that HIV-1 Env incorporation and MA trimerization are functionally connected.

We recently identified a point mutation in MA (62QR) that was able to rescue the Env-incorporation defects imposed by an extensive panel of mutations. Analysis of the 62QR mutant revealed that interactions at the trimer interface were critical to the rescue of Env incorporation defects (Tedbury *et al.* PLoS Path. 2013). Based on the published data, we introduced lysine residues near the trimer interface, which could be cross-linked by the addition of a low concentration of glutaradehyde. This system allowed us to demonstrate the presence of MA trimers in replication-competent virus, both in immature and mature virions. Experiments with interface mutants have revealed a correlation between inhibition of MA trimerization and Env incorporation. Detailed functional characterization of the MA-MA interaction is ongoing with mutants that impair MA folding, MA trimerization or enhance MA trimerization.

Our genetic and biochemical data strongly support the existence of MA trimers in HIV-1 particles and the functional analyses demonstrate the requirement for MA trimerization in Env incorporation. Combining our data with existing MA structures published by the Hill, Sundquist and Barklis groups, we propose that the trimerization of MA is required to relieve the steric hindrance that would otherwise occur between the long cytoplasmic tail of HIV-1 Env and the MA domain of the Gag lattice. Loss of MA trimerization likely causes exclusion of Env from sites of viral assembly. This essential MA-MA interaction may represent a novel target for anti-retroviral intervention.

BACK

T38. Characterization of Novel Mutations in the HIV-1 Env Glycoprotein that Enhance Cell-to-Cell Viral Transfer

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Infectious HIV-1 particle production is driven by the expression of the Gag polyprotein precursor that recruits host cell factors to assist in viral budding. The p6 domain of HIV-1 Gag contains a YPX_nL motif that interacts directly with the ESCRT-associated protein Alix. We previously demonstrated a functional role of p6-Alix binding in HIV-1 particle production and replication by introducing mutations into p6 and characterizing virus replication kinetics. The most striking defect observed was that of a severe delay in virus replication induced by these mutations. To ascertain the nature of this replication defect, we passaged these viruses in culture and selected for resistant viral isolates. Sequencing of the viral revertants revealed novel mutations in Env that not only exhibit a full rescue of the original Gag mutants, but also exhibit replication kinetics faster than those of wild-type HIV-1 in Jurkat T-cells. To explain this phenomenon, we performed assays to characterize the effects of the compensatory mutations on Gag processing, Env incorporation, virus release, and single-cycle infectivity. We demonstrate that these Env mutations do not affect Gag processing, Env incorporation, or virus release efficiency. Some of the mutants are able to decrease fusogenicity and all exhibit impaired single-cycle infectivity as compared to wild type. We concluded that these Env mutations do not enhance cell-free infectivity but instead we propose that they stimulate cell-to-cell viral transmission. Further characterization of these mutants will provide key insights into the role of Env in cell-cell virus transfer.

BACK

T39. Identification of a Novel Structural Element of Gag Important for the Assembly of Immature HIV-1 Particles

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The viral structural polyprotein Gag plays a key role in assembly and release of HIV particles. Once bound to the inner leaflet of the plasma membrane, Gag molecules form a hexameric protein lattice that after incorporation of necessary viral and cellular components buds from the plasma membrane leading to release of immature virions. During virus release, the Gag polypeptide is processed by the viral protease, which results in the formation of mature infectious virions. It was shown that interactions between CA domains, one of the four domains of the Gag polyprotein, are indispensable for formation of the immature Gag lattice. CA consists of two alpha-helical subdomains: the N-terminal (CA-NTD) and C-terminal domain (CA-CTD). Based on the analysis of the recently determined structure of the immature Gag shell of Mason-Pfizer monkey virus, we suggested a novel site for HIV-1 Gag CA-CA contacts in the immature lattice. This site involves CA residues 122-125, a Pro-Pro-Ile-Pro motif in the loop connecting helixes 6 and 7 (the H6-H7 loop) of CA-NTD from adjacent hexamers. We performed alanine-scanning mutagenesis for each residue of this motif and showed that mutations P122A and I124A significantly impaired virus release and disrupted virus replication in a T-cell line. In contrast, mutations P123A and P125A did not impair HIV-1 particle production or virus replication. In addition, we demonstrated by electron microscopy studies that the P122A mutant generated virions that lacked the characteristic cone-shaped central capsid core. We have selected for compensatory mutations that rescue the defects caused by CA P122A and I124A substitutions and will determine the mechanism for this rescue.

Overall, our data suggest that the H6-7 loop of the HIV-1 CA domain is a new structural element necessary for immature Gag lattice formation.

BACK

T40. Elements of Lentiviral p6 Domain Modulating Viral Assembly

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The p6 domain in lentiviral Gag proteins recruits cellular ESCRT machinery to facilitate viral budding. Additionally, it was previously demonstrated that the p6 domain in SIV Gag also recruits clathrin heavy chain (HC) into viral particles. HIV-1 also incorporates clathrin into particles, but through an interaction with its Pol domain. Mutation of the clathrin binding site in SIV p6 inhibits viral replication and also causes a severe defect in assembly when combined with mutations in the TSG101 binding site (PTAP) of p6. Viral assembly appear arrested at an early step in assembly under these conditions. Spherical particle assembly is restored if the the protein ALIX, which binds both HIV-1 and SIV p6, is overexpressed. Because deletion of the entire p6 domain from HIV-1 or SIV Gag does not impair assembly, we sought to identify the elements in SIV p6 responsible for this assembly defect. When wildtype SIV p6 or an SIV p6 with its amino acid sequence scrambled was introduced into HIV Gag the assembly was not impaired. However, when SIV p6 with point mutations in its clathrin and TSG101 binding sites was introduced, the protein was assembly defective and appeared to be targeted by a ubiqutin ligase. Like in the context of SIV Gag, ALIX overexpression restored proper expression and release. We predict that p6 becomes bound by an unknown cellular factor that restricts assembly but can be alleviated by binding of TSG101, clathrin, or ALIX.

BACK

T41. PTAP L Domain-Dependent Modulation of Ca^{2+} Signaling Facilitates HIV-1 Gag Assembly and Budding

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Previously, we reported that a proteomic search for membrane-associated factors that might facilitate ESCRT function in virus budding identified the 1,4,5-inositol triphosphate receptor (IP3R) as differentially associated with HIV-1 Gag containing or lacking an intact PTAP motif. The IP3R protein forms a calcium channel that mediates release of Ca^{2+} from ER Ca^{2+} stores. Channel opening requires IP3R activation that is initiated by the binding of 1,4,5-triphosphate (IP3) that is generated in the cell following hydrolysis of PI(4,5)P_2 by phospholipase C. We subsequently found that Gag release from the plasma membrane (PM) as infectious virus particles requires IP3R function, a finding consistent with observations from several groups, including ours, that raising cytosolic Ca^{2+} enhances viral particle production. Thus, IP3R and the ER Ca^{2+} store are the physiological providers of Ca^{2+} for Gag assembly and release. Specifically, IP3R is required for Gag delivery to and stable association with PM. Here, we show that modulation of ER store Ca^{2+} release and ER store refilling are required components of productive Gag assembly. Cells expressing Gag exhibited a higher level of cytosolic Ca^{2+} originating from the ER Ca^{2+} store than control cells, suggesting that Gag induced the change. This Gag-induced activity required a functional PTAP L domain, the motif in Gag that recruits Tsg101, a component of the ESCRT-1 complex. Consistent with the elevated Ca^{2+} level detected in Gag-expressing cells, stable Gag association with PM was found to require continuous IP3R function. Several lines of investigation suggest that the observed Gag modulation of IP3R channel activity reflects physical proximity, as shown for other IP3R channel modulators. (*i*) TIRF analysis revealed Gag accumulation at ER-PM junctions. (*ii*) Immunoelectron microscopy and indirect immunofluorescence demonstrated that endogenous IP3R is proximal to PM-localized Gag. (*iii*) Reciprocal co-immunoprecipitation from cell lysates suggested Gag and IP3R complex formation. Complex formation was favored when the PTAP motif was intact. Consistent with enhancement of Ca^{2+} release from the ER Ca^{2+} store, we observed an increase in PM PI(4,5)P_2 accumulation, an indicator of store refilling, in cells expressing Gag compared to control cells and this too was promoted when the PTAP motif was intact. Surprisingly, 2-aminoethoxydiphenyl borate (2-APB), an inhibitor of ER store refilling, did not block WT Gag VLP production but reduced budding following disruption of the PTAP motif. We conclude that a functional PTAP L domain, and by inference Tsg101 binding to Gag, confers modulatory activity for both ER store Ca^{2+} release and ER store refilling.

BACK

T42. Temporal and Spatial Organization of ESCRT Protein Recruitment During HIV-1 Budding

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HIV-1 virions assemble at the plasma membrane of mammalian cells and recruit the ESCRT (endosomal sorting complex required for transport) machinery to enable particle release. However, little is known about the temporal and spatial organization of ESCRT protein recruitment. Using multiple-color live-cell TIR-FM (total internal reflection fluorescence microscopy) we observed that the ESCRT-I protein Tsg101 is recruited together with Gag to the sites of HIV-1 assembly, while later acting ESCRT proteins (Chmp4b, Vps4A) are recruited sequentially, once Gag assembly is completed. Chmp4b, a protein that is required to mediate particle scission, is recruited to HIV-1 assembly sites ~ 10s before the ATPase Vps4A. Using two-color super-resolution imaging, we observed that the ESCRT machinery (Tsg101, Alix, Chmp4b/c proteins) is positioned at the periphery of the nascent virions with the Tsg101 assemblages positioned closer to the Gag assemblages than Alix, Chmp4b or Chmp4c. These results are consistent with the notion that the ESCRT machinery is recruited transiently to the neck of the assembling particle and is thus present at the appropriate time and place to mediate fission between the nascent virus and the plasma membrane.

BACK

T43. The Vps4 AAA ATPase Binds ESCRT-III Substrates as an Asymmetric Hexamer

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Vps4 is an AAA ATPase that is part of the multi-component ESCRT (Endosomal Sorting Complexes Required for Transport) pathway, which drives membrane fission during the abscission stage of cytokinesis, the formation of intraluminal vesicles at the late endosome, and HIV budding. Vps4 is recruited to ESCRT-III proteins, which induce membrane deformation by forming a helical polymer within the membrane neck, and uses energy from ATP hydrolysis to release ESCRT-III subunits to their soluble conformation. Vps4 is recruited to ESCRT-III polymers by binding of its N-terminal MIT domain to sequence motifs on ESCRT-III proteins termed MIM (MIT Interacting Motifs). Polymer disassembly is driven by the Vps4 AAA ATPase cassette, which also mediates formation of the active Vps4 oligomer.

Here we show that active wild-type Vps4 comprises an asymmetric hexamer. We also demonstrate a direct interaction between the Vps4 central pore loops and peptides derived from an ESCRT-III subunit that are N-terminal to the MIM, and that this secondary interaction is autoinhibited in the absence of MIM-MIT binding. These findings support a two-step model of Vps4-mediated ESCRT-III lattice disassembly in which initial binding of ESCRT-III MIMs by the MIT domain is followed by substrate binding to the Vps4 ATPase domain and translocation through the central pore of the hexamer, thereby creating a mechanical force that causes release of polymerized ESCRT-III subunits.

BACK

T44. Molecular Modeling of the Spacer Peptide Region of Immature Retroviral Gag

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Retroviruses like Human Immunodeficiency Virus (HIV) encode the Group antigen (gag) polyprotein that is essential during the budding and maturation phases of the virus infective cycle. Gag consists of matrix protein (MA), capsid protein (CA), nucleocapsid protein (NC), and other smaller peptides. For Rous Sarcoma Virus (RSV) and HIV, there is a short spacer peptide (SP) domain in between CA and NC. This SP domain plays an important role in immature retroviral assembly and acts as a molecular switch in stabilizing the immature retroviral lattice. Recent experimental efforts have greatly increased our understandings of the structure of SP, but the structural information of SP at the atomic level remains incomplete.

The last few residues of CA, the SP and the first few residues of NC were recently proposed to coalesce and form a six-helix bundle (6HB) model. Based on the proposed 6HB model, cryo-electron microscopy and computational modeling, we present an improved model of the SP of immature RSV gag. The structural stability of our model was tested through all-atom molecular dynamics (MD) simulations. We show that the C-terminal domain of the immature gag lattice is unstable in the absence of SP. After 100ns of MD simulation, the original 6HB model was distorted and collapsed radially inward. Our 6HB model maintains its 6-fold symmetry through hydrophobic interactions and salt bridges. Additionally, a kink was observed in the middle of the 6HB that is absent in the original 6HB model.

BACK

T45. Associative Interactions of the SP1 Domain of HIV-1 Gag

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Expression of a retroviral protein, Gag, in mammalian cells is sufficient for assembly of immature virus-like particles (VLPs). VLP assembly is mediated largely by interactions between the capsid (CA) domains of Gag molecules but is facilitated by binding of the nucleocapsid (NC) domain to nucleic acid. HIV-1 virus particle assembly in vivo is not impacted when the NC domain is replaced by leucine zippers, even though the VLPs thus produced do not contain significant amounts of nucleic acid. This suggests that the role of nucleic acid in assembly is to concentrate Gag proteins locally. Since the NC domain (nucleic acid binding) and CA domain (assembly determinant) are connected by the SP1 domain, the properties of SP1 were investigated. We recently reported that free SP1 is nearly unstructured in aqueous solution but undergoes a concerted change to a helical conformation when the polarity of the environment is reduced by addition of dimethyl sulfoxide (DMSO), trifluoroethanol, or ethanol. Remarkably, such a coil-to-helix transition is also recapitulated in an aqueous medium at high peptide concentrations. Measurements at different temperatures showed that this transition is entropically driven: it seems likely that at high SP1 concentration, SP1 molecules form helices and associate with each other, leading to the burial of hydrophobic residues. We are now investigating the properties of chimeric constructs consisting of SP1 fused to leucine zippers. These small proteins are helical even under dilute conditions, where free SP1 is unstructured. Most interesting, while leucine zippers form dimers, the chimeric constructs oligomerize into discrete higher-order structures under these conditions. Disruption of the helical register of SP1 in these constructs impairs this higher-order oligomerization. These results suggest that SP1 not only acts as a “sensor” of local concentration and triggers viral assembly, but also participates in specific lateral interactions in the assembly of VLPs.

BACK

T46. MAS NMR Studies of HIV-1 Maturation Intermediates: Investigation of SP1 Conformation in Tubular Assemblies

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A key step in HIV-1 maturation is the cleavage of the Gag polyprotein into its constituent domains.[1] The final step in the Gag cleavage cascade is the severing of the SP1 peptide from CA. Upon cleavage, CA protein condenses to form the capsid shell and an infectious particle. In our recent report, we found SP1 to be unstructured in CA-SP1 tubular assemblies.[2]

In this work, we present new magic angle spinning NMR results on CA-SP1-NC and CA-SP1 tubular assemblies. We examine the conformation of the SP1 peptide in the presence and absence of the NC domain. We analyze the effect of a point mutation in the SP1 peptide that prevents the cleavage of SP1 from CA, thus mocking the effect of maturation inhibitor molecules. We demonstrate that in all cases, high-resolution multidimensional MAS NMR spectra are obtained, which are amenable to detailed structural and dynamics characterization of these assemblies.

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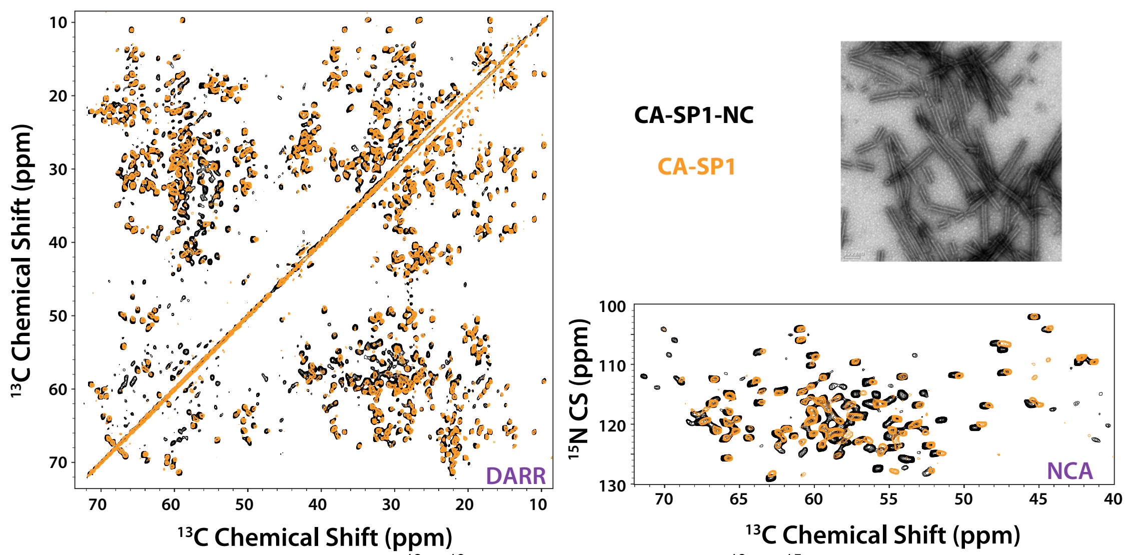

Figure 1. Left: Overlay of homonuclear ^{13}C-^{13}C DARR spectra of U-^{13}C, ^{15}N HIV-1 CA-SP1-NC A92E tubular assemblies (black) with CA-SP1 tubular assemblies (orange). Top right: TEM image for CA-SP1-NC A92E tubular assemblies. Bottom right: Overlay of heteronuclear ^{15}N-^{13}C NCA spectra of U-^{13}C, ^{15}N HIV-1 CA-SP1-NC A92E tubular assemblies (black) with CA-SP1 tubular assemblies (orange).

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BACK

T47. HIV-1 Maturation *In Vitro*

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HIV-1 virions assemble as immature particles containing $Pr55^{gag}$ and $Pr160^{gag\text{-}pol}$ polyproteins. During assembly and budding, the polyproteins are processed by the viral protease into their individual components, resulting in the conversion of the immature lattice into the mature conical capsid. A detailed structure of the mature capsid lattice has been reported, but the structure of the immature $Pr55^{gag}$ lattice is not known. Recent studies of the Mason-Pfizer monkey virus and of HIV-1 reported that the immature lattice of these two retroviruses are structurally distinct from the mature capsid lattice, suggesting that maturation occurs via dissociation of the immature lattice prior to assembly of the mature capsid lattice. To study the structural rearrangements occurring during HIV-1 maturation, we simulated HIV-1 maturation in vitro by digesting detergent-treated immature particles with HIV-1 PR. Processing of $Pr55^{gag}$ by PR was accurate and efficient in the reaction. Cleavage at the CA-SP1 junction was specifically delayed by the maturation inhibitor bevirimat, indicating that the immature cores were of the correct structure for binding the inhibitor. Biochemical analysis of the PR-treated particles revealed dissociation of MA during the reaction; by contrast CA-SP1 and CA remained associated, while cryo-EM analysis of PR-treated particles revealed the formation of assemblies resembling mature capsid structures, suggesting the possibility of reorganization of the lattice rather than complete dissociation and reassociation of the subunits during particle morphogenesis. In addition to studying the mechanism of HIV-1 maturation, this experimental system can be employed to determine the effects of mutations and inhibitors on HIV-1 capsid assembly.

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BACK

T48. Characterization of HIV-1 GagΔ Proteolysis in Vitro at the Capsid/SP1 and SP1/Nucleocapsid Cleavage Sites

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The HIV-1 Gag protein, the principal structural polyprotein, must be cleaved by the viral protease in a regulated and ordered manner to form specific protein cleavage products that will reassemble into the mature core, generating infectious virions. Although the order of individual cleavage events has been described, the mechanisms by which ordered Gag cleavage is orchestrated are unknown. Our lab and others have shown that the rate of certain cleavage events may be regulated by the surrounding structural context of the polyprotein. We are investigating the contextual regulation of cleavage rates at the Capsid/SP1 and SP1/Nucleocapsid cleavage sites utilizing an *in vitro* proteolysis assay with recombinant abridged Gag substrates derived from the NL4-3 strain. Previous work utilized full length Gag derived from the HXB2 reference strain and generated via *in vitro* translation. This previous work showed that, at pH 5, the SP1/NC site is cleaved 20 fold faster than the CA/SP1 site, while SP1/NC cleavage occurs 400 fold faster than CA/SP1 cleavage at pH 7. In our assays, we found that the CA/SP1 site is cleaved slightly faster than the SP1/NC site at pH 5.5 and that the SP1/NC site is cleaved slightly faster than the CA/SP1 site at pH 7.2. We hypothesize that part of the discrepancy in results is due to the difference in the SP1/NC cleavage site sequence between the NL4-3 and HXB2 strains. When replacing the NL4-3 SP1/NC cleavage site with the HXB2 cleavage site residues, we observed a 5-fold increase in the SP1/NC cleavage rate. These results identify important residues that affect the rate of cleavage at the SP1/NC site (P1' and P3'), but still leave a significant difference in the relative rates of cleavage between these two experimental systems.

BACK

T49. Viral Quasispecies and Mutational Analysis Using Next-Generation Sequencing

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The development of resistance against HIV protease inhibitors has contributed to the persistence of the HIV/AIDS epidemic. While the role of drug resistance mutations in protease have been studied comprehensively, mutations in its substrate, Gag, have not been extensively cataloged. Next-generation sequencing has transformed our understanding of the mutational landscape of human pathogens such as HIV and HCV and model systems such as Flock House Virus and Sindbis Virus. I will describe a number of new techniques that combine virological techniques and molecular biology as well as computational analyses of next generation sequencing data, to examine potential co-evolution of HIV-1 Gag and protease from a patient population. The serum or plasma patient samples were obtained when therapy failed to adequately suppress viral replication (generally 1,000 copies/mL), allowing multiple samples to be taken for some patients. By analyzing mutational events such as simple nucleotide mismatches, micro-insertions and deletions, RNA recombination and non-viral RNA packaging, we can gain insights into the functional genetic motifs required for viral replication and the structure-function relationships of protease and Gag.

BACK

T50. Structural Basis of Drug Resistant I50V/A71V HIV-1 Protease and Gag Substrate Coevolution

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Human immunodeficiency virus type-1 (HIV-1) protease is responsible for the processing of Gag and Gag-Pol viral polyproteins to release viral enzymes. As this process is essential for the maturation of HIV viral particles, the protease is an important target for anti-HIV therapy. There are currently nine FDA-approved protease inhibitors (PIs), all of which are competitive inhibitors blocking the substrate-processing active site, and some of them are important components of highly active antiretroviral therapy (HAART).

Under the selective pressure of therapy, the virus accumulates mutations in the protease to impair the efficacy of PIs and confer drug resistance. While the mutant protease has lowered binding affinity to the PIs, it still maintains the ability to cleave its substrates. The selection of such primary mutations in drug resistance to skew the balance between inhibitor binding and substrate processing is effectively explained by our substrate envelope hypothesis. We found that HIV protease recognizes its substrates of diverse sequences by a three dimensional conserved volume shared by different substrates, and drug resistance mutations are more likely to happen at sites where protease residues contact the inhibitors that protrude out the substrate envelope. Within major primary drug resistance mutations, mutation of residue 50 from Ile to Val is commonly observed in patients failing therapy with APV and DRV (two FDA approved protease inhibitors). Residue 50 is located at the flap tip of the flexible loop that controls the access of inhibitors and substrates to the protease active site. Besides conferring resistance to PIs, I50V primary mutation impairs the substrate processing activity of the protease, but this loss in catalytic efficiency is compensated by secondary mutations. A71V is observed in more than 50% of patient sequences with I50V resistance mutation and recovers the protease activity. In addition, several substrate mutations in Gag cleavage sites co-evolve with primary protease mutations, and contribute to protease resistance to the inhibitors.

Substrate mutations Gag L449F, R452S and P453L within the p1-p6 cleavage site are associated with I50V protease mutation in patient sequences. Gag L449F mutation rescues the protease activity by 10 fold, while P453L, although located distal from the catalytic site, causes a 23-fold enhancement. However, the molecular basis for the selection advantage of these co-evolution mutations and how they enhance substrate recognition to confer drug resistance is not clear. In this study, we investigate the structural basis of I50V/A71V protease and p1-p6 substrate co-evolution. We find through a series of co-crystal structures that Gag mutations L449F and P453L enhance van der Waals interactions between the substrate and mutant protease, while R452S results in an additional H bond. Unexpectedly, the P453L substrate mutation causes a conformational change in the protease flap loop, revealing the adaptability of the protease and the molecular mechanism by which this distal substrate change is able to enhance substrate–protease interactions. In addition, our molecular dynamics (MD) simulations reveal that mutations only in protease or only in the substrate expand the active site (increase the distance between 80s loops), while co-evolution restores the distance back to the scale similar to WT protease-substrate complex.

BACK

T51. Deep Sequencing of Many Drug Resistant HIV Patients Reveals Correlated Mutation Patterns in Gag and Protease

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While the role of drug resistance mutations in HIV protease has been studied comprehensively, mutations in its substrate, Gag, have not been extensively cataloged. Using deep sequencing, we sequenced a uniquely large collection of longitudinal viral samples from 93 patients who have been treated with therapies containing protease inhibitors (PIs). Due to the large sequence coverage within each sample, the frequencies of mutation at individual positions can be calculated with high precision. We use this information to characterize the variability in the Gag polyprotein and its effects on PI-therapy outcomes. Additionally, because the single-site mutation frequencies in each sample tend toward either zero or one, it is possible using an approach we developed to estimate tight bounds on the two-site bivariate probabilities and then to examine covariation of mutations between two sites using deep sequencing data. We find that mutations in the matrix and p6 proteins contribute to continued therapy failure and play large roles in the network of strongly correlated mutations in Gag polyprotein and between Gag and protease. Although covariation is not direct evidence of structural propensities, we find the strongest correlations between residues on capsid and matrix of the same Gag protein are often due to structural proximity. This suggests that some of the strongest inter-protein Gag correlations are also due to structural proximity. The strong covariation between residues in matrix and capsid at the N terminus with p1 and p6 at the C terminus is consistent with residue-residue contacts between these proteins at some point in the viral life cycle.

T52. Structural Insights into Substrate Co-Evolution

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Resistance to various Human Immunodeficiency Virus -1 (HIV-1) protease inhibitors (PIs) has made it increasingly difficult to treat HIV/AIDS patients. The virus accumulates mutations within the protease (PR) that render the PIs less effective. Occasionally, cleavage site mutations arise and are often correlated with the protease mutations contributing to the overall resistance to PIs. Thus far, little is known regarding the co-evolution of PR-substrates to substrate recognition, cleavage and resistance. In this study, the co-evolution of p1-p6 cleavage site with the nelfinavir (NFV)-resistant D30N/N88D protease mutations was investigated. Structures of WT and NFV-resistant HIV-1 protease in complex with p1-p6 substrate peptide variants with L449F and/or S451N mutations were examined with respect to alterations in vdW interactions, hydrogen bonds and fit within the substrate envelope. Mutations in PR and/or the substrate alter interactions between PR D30 and p1-p6 cleavage site residues, which are compensated by the co-evolving L449F and S451N mutations in the p1p6 cleavage site. This study demonstrates that PR-substrate interactions at different p1-p6 residues are altered in a correlated manner. This interdependency in the PR—p1-p6 interactions is likely to affect the overall fit of the substrate within the substrate envelope and play a role in substrate recognition and cleavage in the presence of PR resistance mutations. Additionally, preliminary Gag processing studies with WT and NFV-resistant HIV-1 protease reveal that this co-evolution may alter the rate of p1-p6 cleavage site.

BACK

T53. Probing Molecular Interactions of Protease with Small Molecules and Gag Cleavage Junctions

Tiefenbrunn, T[1]., Forli, S.[1], Happer, M.[2], Gonzalez, A[3]., Baksh, M. M.[4], Chang, M. W.[5], Tsai, Y.-S.[3], Lin, Y.-C.[2], Perryman, A. L.[1], Rhee, J.-K.[4], Torbett, B.E.[5], Olson, A. J.[1], Soltis, M.[3], Elder, J. H.[2], Finn, M. G.[4], Stout, C. D.[1]

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A crystallographic fragment-based screen against PR identified two surface sites, the flap site and the exosite, that are potentially important for protein-protein interactions with Gag and are alternatives to the active site for drug design (1). Cocrystals of indole-6-carboxylic acid and 3-indolepropionic acid binding in the flap site were obtained with apo PR with closed flaps (2). A larger BSI phase change was observed for binding to apo PR vs. pepstatin:PR, indicating a larger conformational change upon compound binding in solution, suggesting that compound binding forces the flaps closed.

To find additional hits, a brominated fragment library was screened against a novel $I4_122$ form of W6A PR. Of a library of 68 compounds, one hit was identified in the flap site and one in the exosite. An additional 9 bromine binding sites were found in each site with a strong Br anomalous peak but weak compound density. Br sites provide insight into the nature of the binding pockets, and, for the flap site, show a clear direction for future fragment growth. In the exosite, the movement of Lys14 upon compound binding has motivated computational studies utilizing this different conformation of the exosite as a binding pocket.

Based on prior fragment hits, computational studies in the Olson group identified 1-(4-methylphenyl)sulfonyl-3-(1,3-thiazol-2-yl)urea (C6) as a potential exosite binder. C6 binds to PR as indicated by DSF and BSI assays with 6.5 ºC stabilization and 0.96 +/- 0.11 μM affinity, respectively. C6 has been co crystallized in two crystal forms of TL-3:PR, conserving interactions observed for other fragments. The observed binding mode of C6 involves a portion of the molecule being solvent exposed, suggesting modes for fragment evolution to more closely bind in the exosite pocket of PR.

In order to visualize detailed, specific interactions of PR with its cleavage junctions in the context of Gag, simplified constructs containing a single cleavage junction, a previously-crystallized Gag domain, and a His tag to ease purification have been designed. Several constructs have been expressed, purified, and refolded, including mutants to decrease protease activity at the cleavage junction. Size exclusion chromatography and native gel electrophoresis have been utilized to assay for complex formation with a number of protease variants. Such Gag component complexes address PR binding to a cleavage site while potentially interacting with an adjacent Gag domain via protein–protein contacts. In general, this approach affords an opportunity to address key structural aspects of PR – Gag interaction by using complexes of smaller polypeptides more amenable to crystallization.

BACK

T54. Deep Sequencing Analysis Reveals Novel Pathways to Protease Inhibitor Resistance: In Vitro Selection of Resistance Mutations to New Potent HIV-1 Protease Inhibitors

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We carried out in vitro selections with Darunavir (DRV) and 8 potent PI-derivatives of DRV using with a mixture of 26 variants, each containing single PI-resistance mutation. Selection was carried out to increase the inhibitor concentration to between 1,000- and 10,000-fold over the IC50. Using the Primer ID-based paired-end MiSeq platform, mutations in the protease region were analyzed at four time points. We observed unique patterns of resistance mutations associated with the structure of the inhibitors used for selection, based on modifying two positions in the PIs, R1 (isobutyl) and R2 (amine) in DRV. Either isopentyl or isohexyl was used to replace R1 and one of five different groups was used to replace R2. We found three distinct resistance patterns within the active site depending on the nature of R1 and R2 positions. When R1 was isopentyl (relatively small), R1 appears to determine resistance patterns regardless of R2 moiety, driving the V82I/I84V resistance pathway (UMASS-1, 2, 4, and 5). When R1 was isohexyl (relatively large), R2 appears to determine resistance patterns. In this case, R2 containing an oxygen drove a resistance pathway using I50V/L63P/I85V (UMASS-7, 8, and 9), and R2 having an amine, the same as in DRV, followed the same V82F/I84V pathway as that obtained with DRV. Many of the mutations outside of the active site appeared as linked pairs closely spaced in the protease sequence suggesting one modulated the effect of its nearby partner. Also, many mutations were seen that were not included in the starting mixture, suggesting sequence diversity in the culture is not limiting. Surprisingly, the most abundant mutant, typically containing 4 to 10 mutations, never reached an abundance in the culture of greater than 25% at the final passage. The remaining sequences were a wide array of subsets of the most abundant sequence including the maintenance of the wild type sequence less than 2% in each culture. Taken together, out results demonstrate that even subtle chemical changes in a protease inhibitor can result in the selection of resistance using significantly different mutational pathways and selection in culture appears to occur with a large population size that includes massive levels of recombination.

BACK

T55. The Fitness Landscapes of Drug-Resistance in HIV Protease

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Mutations responsible for resistance to first- and second-generation HIV protease inhibitors are found predominantly within the 'substrate envelope' where the inhibitors bind. However, resistance mutations to third-generation inhibitors are found spread throughout the protease structure, conferring resistance by mechanisms that are not currently well understood. The complicated pathways that lead to resistance require thorough analysis to deconstruct. EMPIRIC (Exceedingly Methodical and Parallel Investigation of Randomized Individual Codons) is a robust method for sampling all possible single amino acid substitutions (1-, 2-, or 3-nucleotide substitutions at each codon) that utilizes deep sequencing to quantify the frequency of each mutation within a specific genetic background. Using a high-throughput yeast reporter screen linking protease activity to GFP expression, the effect of each amino acid substitution on protease activity can be determined in the absence and in the presence of inhibitor. EMPIRIC analysis will identify mutations resistant to inhibitor and assess the contributions of individual mutations to drug resistance networks. The flexibility to change the genetic background used in EMPIRIC will allow us to monitor how different resistance mutations affect the accessibility of sequence space, providing detailed maps of the paths leading to resistance. The resistance profiles identified through the screens will be assayed in full-length HIV to determine their fitness within the context of the viral replication cycle. The mutational fitness maps can be used to inform future inhibitor designs by identifying the mutational lynch pins that underlie resistance networks.

BACK

T56. Characterizing Conformational Sampling and Dynamics of HIV-1 Protease via EPR Spectroscopy and NMR Spectroscopy

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We have been utilizing pulsed EPR spectroscopy and solution NMR methods to characterize the effects that natural polymorphisms and drug pressure selected mutations have on the conformational sampling and backbone dynamics of HIV-1 protease. Our work suggests a hypothesis by which drug-pressure selected mutations combine to induce weakened protein-inhibitor interactions. Specifically, we have found that mutations combine to stabilize more open-like states of HIV-1 protease. Conformational sampling is determined from distance measurements using pulsed EPR spectroscopy. More recently we have been combining backbone relaxation measurements via solution NMR spectroscopy with results from our EPR investigations. Results are showing that mutations that stabilize protein and flap conformations that are open-like, being wide open or more of an asymmetric curled open, also have increased backbone dynamics. In contrast, mutations that stabilize a more closed-like flap conformation have reduced backbone dynamics, particularly in the flaps. Recent EPR and NMR results will be presented and discussed.

T57. REdiii: A Pipeline for Automated Structure Solution

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High-throughput crystallographic approaches require integrated software solutions to minimize the need for manual effort. REdiii is a system allowing fully automated crystallographic structure solution by integrating existing crystallographic software into an adaptive and partly autonomous workflow engine. The program can be called after collecting the first frame of diffraction data and is able to perform processing, molecular replacement phasing, chain tracing, ligand fitting and refinement without further user intervention. A set of smart presets for each software component allows efficient progress with high quality data and known parameters. The adaptive workflow engine can determine required modifications to these parameters and choose alternative software in case the preconfigured solution is inadequate. This integrated pipeline is targeted at providing a comprehensive and efficient approach to screening for ligand-bound co-crystal structures while minimizing repetitiveness and allowing a high-throughput scientific discovery process.

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T58. Understanding Molecular Recognition in HIV Protease and Integrase Using Binding Free Energy Methods

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Molecular docking is a powerful tool used in drug discovery, and structural and chemical biology for predicting the structures of ligand-receptor complexes. Docking is often performed to screen a very large number of compounds which are ranked using scoring functions. However, the accuracy of docking calculations can be limited by factors such as the neglect of the reorganization in the scoring function; as a result ligand screening can produce a high rate of false positive hits.

In the first part of this work we apply absolute binding free energy methods to reduce the false positive rate and improve the docking results. Although free energy methods still have difficulty in accurately rank ordering binders, we believe that they can be fruitfully employed to separate binders from non-binders.

We study a set of ligands that dock favorably to a newly discovered allosteric site on the flap of HIV-1 protease. Top ranked protein-ligand complexes from AutoDock are used as the starting point for the free energy calculations. Two methods, the recently developed Binding Energy Analysis method (BEDAM) and the standard Double Decoupling Method (DDM) were employed to compute the binding free energies for 23 ligands, including 3 confirmed actives, 7 likely binders, and 13 false positives. The majority of the false positives are ranked more favorably by docking score than the known binders. Free energy calculations correctly identified most of the false positives (84.6 %) and recovered all the confirmed binders. The results show a gap averaging ~ 4 kcal/mol separating the binders and the false positives. Four out of seven likely hits are predicted to bind at the flap site. The free energy simulations also provided important structural and thermodynamic insights on binding. Our calculations suggest that the intermolecular salt bridge between the Arg57 and the carboxylate in the ligand 1F1/1F1N makes the dominant contribution to binding. Decomposition of the binding free energies suggests improving the treatment for the desolvation penalty associated with the unfulfilled polar groups could reduce the false positives rate in docking.

In the second part of the study, we show how free energy calculation can elucidate the molecular mechanism for a drug resistance mutation in HIV Integrase (IN). The Integrase/LEDGF site inhibitor BI-D binds to both wild-type IN and H171T mutant with the identical pose, yet the latter shows resistance. Free energy calculations suggest that mutation decreases the affinity without changing the binding mode: the doubly protonated or N-d protonated forms of the H171 side-chain forms an unusually strong hydrogen bond network with BI-D, compared with T171. Free energy calculations also show that the H171T mutation has a similar but smaller effect on the binding affinity of LEDGF, in agreement with experiments. Furthermore, the calculations suggest that this differential effects on the BID and LEDGF binding is due to the fact that the H171T mutation perturbs the hydrogen bonding network involving the indole ring of the H171 side-chain and the t-butoxy group in the BI-D; since the LEDGF binding loop doesn't have such a t-butoxy group, the H171T mutation has a smaller effect on LEDGF binding.

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T59. Incorporating Bridging-Waters into Lead Discovery Using Molecular Footprints

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In many systems of biological importance, water molecules at specific locations are observed to be involved in bridge-type hydrogen bonding which helps to mediate protein-ligand binding. Well-known examples include HIV-1 protease and EGFR. In terms of rational drug design, one possible strategy to enhance binding affinity in such systems is to design ligand analogs that contain functionality that displace and/or mimic the interactions made by the bridging waters. This goal of this work is to identify, quantify, and analyze a series of water-mediated interactions through comparison of molecular footprints (per-residue energy decomposition) generated with and without specific waters present. The goal is to provide new methods for lead discovery (virtual screening) in which solvated footprints can be used a reference to identify new compounds that mimic the interaction patterns made by the reference. Results for several systems will be presented, including HIV-1 PR, for which the well-known class of cyclic urea analogs was designed to displace (replace) the key flap water.

T60. Understanding the Structural Basis of Drug Resistance in Influenza Neuraminidase

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Although vaccines and antiviral medications are available for influenza prophylaxis and treatment, influenza infects over 24 million people in the United States each year, causing over 200,000 hospitalizations and 40,000 deaths. Influenza neuraminidase (NA) is a viral sialidase on the surface of the influenza virion that releases budding virus from the surface of infected cells, and it is a primary antiviral target in the treatment of influenza. Two subtypes of NA predominate in humans, N1 and N2, but different patterns of drug resistance have emerged in each subtype even though the active sites of these NAs are highly homologous. Our laboratory studies drug resistance in viral proteases, and we found that natural substrates of HIV and hepatitis C proteases fill a conserved enzyme-specific volume when bound to the active site, known as the *substrate envelope*. Furthermore, primary drug resistance mutations occur where inhibitors protrude beyond the *substrate envelope,* and our laboratory established this paradigm as the *substrate envelope hypothesis.* To provide a framework for understanding the structural basis of subtype specific drug resistance mutations in NA, I am completing a comprehensive analysis of existing crystal structures, models, and molecular dynamics simulations incorporating the *substrate envelope hypothesis* to provide atomic level insight into the impact of drug resistance mutations on protein structure and dynamics.

T61. Examining the Genetic Barrier of GRL008, a Novel HIV-1 Protease Inhibitor Designed Using Crystallographic Solvent Mapping Technique

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Human immunodeficiency virus type-1 (HIV-1) protease has been a critical drug target for designing protease inhibitors (PIs). Understanding the resistance mechanisms as well as development of novel PIs that are potent against multi-PI-resistant strains of HIV-1 is crucial. Recently, a structure-based drug design approach using crystallographic solvent mapping has been employed to design, synthesize and evaluate a novel PI, GRL008 (Yedidi et al., Antimicrob Agents Chemother. 2013). X-ray crystal structure of wild type HIV-1 protease (PR_{WT}) in complex with darunavir (DRV-the latest FDA-approved PI) (PDB ID: 4HLA) was used as a template. GRL008 showed potent inhibition of PR_{WT} (K_i: 8.9 pM) compared to the parent compound, DRV (K_i: 16 pM). In antiviral assays using human MT-4 cells, GRL008 was potent (50% effective concentration, EC_{50}: 0.038 μM) in inhibiting not only the wild type HIV-1 (HIV_{WT}) viral replication but also the replication of a multi-PI-resistant strain of HIV-1 derived from a clinical patient isolate (HIV_{A02}) with an EC_{50} of 0.029 μM. Further, GRL008 has recently been shown to inhibit a DRV-resistant strain of HIV-1 at DRV selection passage 20 ($HIV_{DRV}{}^{R}{}_{P20}$) suggesting that it has a higher genetic-barrier against multi-PI-resistant HIV (Yedidi et al. Antimicrob Agents Chemother. 2014, *in press*). Crystal structures of GRL008 in complex with either PR_{WT} or PR_{A02} showed identical binding profiles for GRL008 supporting its antiviral potency. Thus our present structure-function studies reveal GRL008, as a potent PI with a desirable genetic barrier against HIV_{WT}, HIV_{A02} and $HIV_{DRV}{}^{R}{}_{P20}$.

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T62. The Catalytic Activity of the HIV-1 Protease is Enhanced by RNA

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In the maturation step of the HIV-1 lifecycle, the virus-encoded protease (PR) must process the structural polyprotein Gag into its constituent parts to generate mature, infectious virus particles. Previous reports suggested that proteolytic cleavage of the p15NC intermediate is enhanced by the presence of RNA. As p15NC contains the principle RNA-binding domain (NC), the basis of this effect was thought to result from an interaction between substrate and nucleic acid. In an effort to elucidate the mechanism of RNA-dependent acceleration of p15NC cleavage, we utilized a two-substrate proteolysis system to study the rate of p15NC cleavage relative to an internal control (HIV-1 MA/CA). Our results demonstrated that both substrates were cleaved more rapidly in the presence of RNA. In single-substrate assays, HIV-1 MA/CA processing increased by >100-fold in reactions including heteropolymeric RNA at pH 6.5. Furthermore, we observed a 15-fold increase in cleavage rate for a fluorogenic peptide substrate in the presence of RNA, demonstrating that RNA-dependent enhancement is substrate-independent. Assays employing 18-to-21mer DNA or RNA oligonucleotides showed that oligos containing a tetra-G sequence were preferentially capable of accelerating HIV-1 MA/CA cleavage. A 4-mer comprising the GGGG sequence verified that the tetra-G motif is sufficient for accelerating HIV-1 PR activity. Longer RNAs increase the magnitude of the rate acceleration, though tRNAs appear exempt from this relationship. To investigate whether increased dimer stability (i.e. lower rates of monomer dissociation) accounted for the observed acceleration of PR activity, we performed reactions with a tethered dimer version of the HIV-1 PR. Cleavage of HIV-1 MA/CA by the tethered dimer PR was accelerated equivalently by RNA, indicating reduced dissociation is not the mechanism of the observed increase in catalytic activity. In addition to the HIV-1 Subtype B PR, both Subtype C and AE PRs displayed a similar RNA-dependent enhancement of catalytic activity, although the activity of the HIV-2 PR was not affected in reactions supplemented with RNA. In summary, we have demonstrated that RNA-dependent enhancement of HIV-1 PR activity is a substrate-independent phenomenon. Furthermore, our results suggest a direct interaction occurs between the HIV-1 PR and nucleic acid, with a sequence preference for a tetra-G motif, and that this interaction enhances HIV-1 PR activity. These results raise the possibility that protease cleavage during assembly is regulated in part by the juxtaposition of the viral protease to viral genomic RNA and/or virion-packaged tRNAs.

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T63. High Resolution Structure of HIV-1 Capsid Assembly

Gongpu Zhao[1,2], Xueming Li[3,4], Chuang Liu[1,2], Jiying Ning[1,2], Randall T. Schirra Jr.[1,2], Angela M. Gronenborn[1,2], Yifan Cheng[3] and Peijun Zhang[1,2]

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The human immunodeficiency virus 1 (HIV-1) capsid protein (CA) contains two structural domains that are connected by a flexible linker and can assemble into a distinct cone shaped capsid. The mature HIV-1 capsid plays a major role in host-pathogen interactions that regulate the early stages of HIV-1 replication. Many host factors such as CypA, CPSF6, MxB, TRIM5α and TRIM-Cyp have been reported to directly interact with HIV-1 capsid and modulate viral infectivity. We have previously determined the CA tubular assembly to 8.6 Å using cryoEM and build an all-atoms model of the complete capsid comprising 3 million atoms by molecular dynamics simulations. Exploiting the recent advance in direct electron detection, we have now reconstructed three cryoEM density maps of CA tubular assemblies from (-12, 11), (-13, 12) and (-13, 11) helical symmetries at 7.0, 7.7 and 7.8 Å resolution, respectively. Averaging the asymmetric subunits from three data sets yielded an improved density map at 5.9 Å resolution. The resulting structure clearly displays bulky side chain densities, helix grooves and connecting loops, which enable more accurate molecular modeling. Comparing the detailed assembly interfaces in the three structures will further shed light on the mechanisms by which CA molecules accommodate the variable curvature of mature conical capsid.

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T64. Coarse-Grained Molecular Simulations of HIV-1 Capsid Protein Self-Assembly

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The HIV-1 capsid protein (CA) is enzymatically cleaved from Gag polypeptide molecules during HIV virion maturation. Subsequent to cleavage, the capsid protein self-assembles into a characteristic cone-shaped viral “capsid” which is a necessary feature of mature and infectious virions.

The mature virion environment is complicated, containing not only many copies of the capsid protein but also various other viral proteins, RNA, and assorted molecules captured from the cytoplasm of the host cell. This complexity, in combination with the relatively large number of proteins needed to construct a viral capsid, makes “coarse-grained” (CG) molecular simulation an attractive technique for the study of capsid self-assembly. We investigate the self-assembly properties of a coarse-grained computational model of the HIV-1 capsid protein, including the effects of a model molecular crowding agent at physiologically relevant molecular densities.

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T65. Atomistic Insights of the HIV Capsid from Molecular Dynamics Simulations

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The HIV capsid is large, containing about 1,300 proteins with altogether 4 million atoms. Although the capsid proteins are all identical, they nevertheless arrange themselves into a largely asymmetric structure. The large size and lack of symmetry pose a huge challenge to studying the chemical details of the HIV capsid. Simulations of 64 million atoms for over 1 micro-second allow us to conduct a comprehensive study of the physical properties of the entire HIV capsid including electrostatic potential, all-atom normal modes, as well as the effects of the solvent (ions and water) on the capsid. The results from the simulations reveal critical details of the capsid protein with important implications for assembly, uncoating and nuclear import.

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T66. Backbone Dynamics in HIV-1 Capsid Protein Assemblies by Hybrid MAS NMR/MD Approach: Insights Into Conformational Plasticity and Interactions with Cyclophilin A

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Structural plasticity of HIV-1 CA is a necessary prerequisite for the protein's assembly into pleiomorphic capsid cones. As shown in our previous work, inherent mobility of the CA protein is connected to its structural plasticity. In this study, we examine residue-specific internal dynamics in assembled CA and its role in the CA's interactions with the host protein Cyclophilin A (Cyp A). CypA binds CA and facilitates HIV-1 replication by an unknown mechanism. We pursued a hybrid magic angle spinning (MAS) NMR / molecular dynamics (MD) approach to examine the backbone mobility of CA assemblies free and with CypA bound, focusing on the backbone motions occurring on the timescales of 10^{-9}~10^{-6} s.

The tubular CA assemblies and CA/CypA complex yield unprecedented high-resolution MAS NMR spectra, permitting their structural and dynamics analysis at atomic resolution. The backbone ^{1}H-^{15}N and ^{1}H-^{13}C dipolar order parameters (DOP) recorded with RN-symmetry sequences in 3D chemical shift resolved experiments, indicate unusually high mobility in the CypA-binding loop region belonging to the N-terminal domain of CA. Remarkably, this mobility is sequence dependent, and the CypA loop dynamics is strongly attenuated in the CypA escape mutant A92E and upon binding of CypA to the assemblies. Dipolar order parameters extracted from the 100-ns MD trajectories are in excellent agreement with the experiments. The results suggest a connection between the inherent dynamics of CA and its interactions with CypA. The hybrid MAS NMR/MD approach established in this work is thus a powerful strategy for atomic-level analysis of dynamics in HIV-1 protein assemblies.

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T67. Structure of Mature HIV-1 Capsid Assembly in Complex with Cyclophilin A

Chuang Liu[1,2], Juan R. Perilla[3], In-Ja Byeon[1,2], Tatyana Polenova[2,4], Chang-Hyeock Byeon[1,2], Jiying Ning[1,2], Jinwoo Ahn[1,2], Chris Aiken[2,5], Angela M. Gronenborn[1,2], Klaus Schulten[3] and Peijun Zhang[1,2]

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Host cell factor cyclophilin A (CypA) plays an important role in modulating HIV-1 capsid function. Interestingly, several host cell proteins, including TRIMCyp and NUP358, contain CypA as a modular domain and interact directly with viral capsid using the domain. The binding of the host protein CypA to the viral capsid is important for infection, yet the structural effects of this binding have not been identified. We determined the cryoEM structure of CypA in complex with an HIV-1 capsid assembly at 8 Å resolution. The density map displays a non-random, selective binding of CypA along the most curved helical direction, forming a bridge directly above the CA CTD-CTD dimer interface cross the adjacent CA hexamers. CryoEM structure-based modeling and large scale all-atom molecular dynamics simulations unexpectedly revealed that one CypA molecule simultaneously interacts with two CA molecules, one of these through a non-canonical novel interface. The individual residues critical for the interactions were further identified and confirmed by solution and solid state NMR spectroscopic studies. Our combined cryoEM, computational and NMR studies provide mechanistic insights into the functional role of CypA in modulating capsid uncoating and viral infectivity, and our structure further highlights the novel CypA and CA interface as an attractive therapeutic target for pharmacological intervention.

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T68. Structural Characterization of HIV-1 Capsid Protein Assemblies Free and in Complex with Cyclophilin A Using Magic Angle Spinning NMR

Manman Lu[1,2], Guangjin Hou[1,2], Huilan Zhang[1,2], Christopher L. Suiter[1,2], Rupal Gupta[1,2], Mingzhang Wang[1,2], Jinwoo Ahn[2,3], Chang Byeon[2,3], In-Ja L. Byeon[2,3], Angela M. Gronenborn[2,3], Tatyana Polenova[1,2]

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The capsid protein (CA) of human immunodeficiency virus 1 (HIV-1) assembles into a cone-like structure comprised by ~1200 copies of CA,[1,2] to enclose the viral RNA genome and a small complement of proteins during viral maturation. The host protein Cyclophilin A (CypA) binds CA and facilitates HIV-1 replication by an unknown mechanism. In this work, we conducted structural studies on CA assemblies using magic angle spinning (MAS) NMR spectroscopy. We have collected intramolecular and intermolecular distance restraints in sparsely and differentially labeled CA assemblies using a suite of homo- and heteronuclear correlation experiments. In order to elucidate the mechanisms of CA-CypA interaction, we conducted titration experiments of CA and CypA using varying CA/CypA ratios. MAS NMR spectra of CA/CypA assemblies exhibit remarkably high resolution, permitting their atomic-level structural analysis (Figure 1). As the ratio of CA/CypA increases, multiple chemical shift perturbations were observed, indicating conformational changes of CypA upon binding to CA assemblies. From these perturbations, the binding interfaces are inferred.

This work was supported by the National Institutes of Health (NIH Grant P50GM082251 from NIGMS). We acknowledge the support of the National Science Foundation (NSF Grant CHE0959496) for the acquisition of the 850 MHz NMR spectrometer at the University of Delaware.

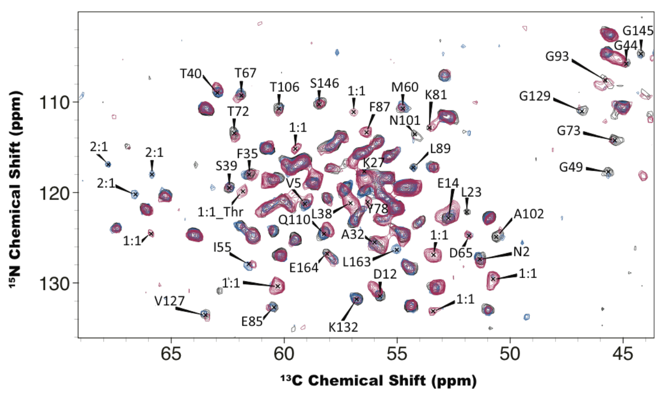

Figure 1. The superposition of the NCA spectra of CypA in complex with CA assemblies at CA/CypA ratio of 4:1 (black), 2:1 (blue) and 1:1 (maroon). Spectra were acquired at 19.9 T. Chemical shift perturbations are labeled in the spectra.

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T69. An Essential Role of INI1/hSNF5 in HIV-1 Post-Transcriptional Mechanisms Leading To Assembly

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INI1/hSNF5 is a HIV-1-specific host protein that binds to HIV-1 integrase (IN) in the context of Gag-Pol and is incorporated into the virions. Expression of a minimal IN-binding domain of INI1 (termed S6) transdominantly inhibits HIV-1 (but not SIV) particle production (*Yung et al Nat. Med. 2003 and J.Virol 2005*). INI1 and IN both associate with Sin3a associated protein, 18 KD (SAP18), which is also incorporated into HIV-1 virions (*Sorin et al PLoS Path. 2009*). Human cells lacking INI1 exhibit a block in HIV-1 particle production, which is restored by the expression of INI1 in these cells (*Sorin et al Retrovirology 2006*). These results suggested that INI1 is required for HIV-1 late events. However, the exact of role of INI1 in HIV-1 late events remained obscure.

To determine the role of endogenous INI1/hSNF5 and INI1-SAP18 interaction to HIV-1 replication, we have analyzed the requirement for INI1/hSNF5 by using three different approaches. We analyzed HIV-1 late events: (i) in the presence of SAP18-interaction defective (SID)-INI1 mutants; (ii) in *INI1-/-* human cells deleted of *INI1* gene; and (iii) by siRNA-mediated knock-down of *INI1* in 293T cells. Expression of SID-INI1 mutants in 293T cells selectively inhibited HIV-1 (but not SIV) particle production (>10,000 fold), similar to that of S6. Pulse-chase analysis indicated that SID-INI1 mutants inhibited post-translational Gag/Gag-Pol stability and processing. Immunofluorescence analysis indicated that SID-INI1 mutants dramatically inhibited intra-cellular trafficking of Gag/Gag-Pol to the membrane.

To establish that INI1 is required for Gag/GagPol stability and intracellular trafficking, we analyzed the HIV-1 late events in MON (*INI1-/-*) cells lacking INI1. In the absence of endogenous INI1, HIV-1 particle production, intracellular p24 levels, and particle release efficiency were highly reduced in MON cells. Furthermore, in the absence of INI1, the Gag/Gag-Pol trafficking to the membrane was dramatically inhibited. On the contrary, when INI1 was introduced into *INI1-/-* MON cells, the intracellular p24 level, particle production, particle release efficiency, and Gag/Gag-Pol trafficking to the membrane were restored. Finally, siRNA-mediated knock down of INI1 resulted in a reduction in the HIV-1 particle release as measured by intra-cellular and virion-associated p24. We are currently investigating the effect of knock-down of INI1 on Gag/Gag-Pol intracellular trafficking in 293T cells. Our studies, for the first time, establish that an IN-binding host protein is specifically required for HIV-1 Gag/Gag-Pol stability and intracellular trafficking in human cells and that lack of INI1/hSNF5 leads to defects in assembly events. Thus, targeting IN-INI1 interaction is a novel strategy to impair post-transcriptional events to potently inhibit HIV-1 replication.

BACK

T70. Inhibition of HIV-1 Particle Maturation by Allosteric Integrase Inhibitors

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Integration is an essential step of HIV-1 replication and the active site of the enzyme responsible, integrase (IN), is an established drug target to prevent the onset of acquired immunodeficiency disease (AIDS). Viruses resistant to active site IN strand transfer inhibitors (INSTIs) arise within treated patients; therefore, the development of inhibitors that bind to novel sites on IN would be highly beneficial, as they should be effective against current INSTI-resistant strains.

A promising class of allosteric IN inhibitors (ALLINIs), 2-(quinolin-3-yl) acetic acid derivatives, engage the IN catalytic core domain dimerization interface at the binding site for the host integration co-factor LEDGF/p75. ALLINIs enhance higher-order oligomerization of IN, indicating that disregulated IN multimerization underlies ALLINI functionality. ALLINI potency is interestingly accounted for during the late phase of HIV-1 replication in a manner that is independent of the level of LEDGF/p75 expression. Virions produced from cells treated with ~25-100 EC_{50} units of various ALLINI compounds fail to properly mature. The normally electron-dense conical core is converted to an eccentric phenotype where the electron-dense material is instead situated between a translucent core and the viral membrane. In subsequent infections, ALLINI-treated viruses are defective for both reverse transcription and integration.

Virions produced in cells treated with doses as low as twice the EC_{50} value revealed the majority of viral cores converted to the eccentric phenotype, indicating that inhibition of virus particle maturation indeed underlies ALLINI potency. We have additionally shown that ALLINI treatment phenocopies ΔIN HIV-1 morphology via use of a vpr-IN trans-complementation of ΔIN HIV-1 viruses. Lastly, we have characterized the drug-induced virus particle defect through the use of cryo-electron tomography (cryo-ET). The nucleic acid content of normal core-associated and eccentric electron densities was probed by bubblegram imaging, a cryo-ET technique that detects protein in dense nucleoprotein complexes through its sensitivity to radiation damage.

BACK

T71. HIV-1 IN Mutants Defective for INI1/hSNF5 Binding Exhibit Impaired Particle Morphology, Reverse Transcription and Integration

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INI1/hSNF5 (Integrase interactor 1) is a component of the first IN-binding protein isolated. Genetic and biochemical studies indicate that INI1/hSNF5 influences multiple stages of HIV-1 replication. Transdominant mutants of INI1 inhibit HIV-1 replication and virions produced in INI1-/- MON cells are defective for infectivity. INI1 as well INI1-associated protein such as SAP18 are specifically incorporated into HIV-1 virions. INI1 also influences integration *in vitro* and LTR transcription *in vivo*.

To further understand the role of INI1, we isolated a panel of INI1-Interaction-Defective (IID)-IN mutants using reverse yeast two-hybrid system. IID-IN mutations were mapped on solved crystal structures of IN and mutations of residues with high surface accessibility on IN (S147G, Q137R, K71R, H12Y, K111E, D202G) were further characterized using virological assays. The panel of IN* mutants were incorporated into HIV-1NL4-3 and tested for replication capacity. IID-IN mutants were defective for replication compared to wild type in CEM-GFP cells. The defect in replication of IID-IN mutants correlated to their defect in interaction. Those mutants highly defective for INI1-interaction were highly impaired for replication and vice versa. The mutants were then incorporated into HIV-Luc and tested for their effect on early events in a single cycle infection assay. Consistent with the results of multi day replication assay, highly defective mutants (Q137R, H12Y, D202G) were 10-100 fold defective and partially defective mutants (K71R, S147G, K111E) were 2-5 fold defective in the HIV-Luc assay. Further analysis indicated that the IID-IN mutants that map to core domain show impaired particle morphology and post entry stages of replication including reverse transcription and integration. None of the IID-IN mutants were significantly defective for binding to LEDGF, GEMIN or RT.

The above analysis indicated a block at or prior to reverse transcription in highly defective mutants. These mutants were localized in the core domain of IN. Our analysis indicates that INI1 binds to both core-domain as well as C-terminal domain of HIV-1 IN. To segregate various functions of INI1 on HIV-1 replication, we also tested the ability of C-terminal domain mutants to bind to INI1. We found that one of the well-characterized C-terminal domain IN mutant does not bind to INI1 and SAP18 but is proficient in binding to LEDGF, GEMIN and RT.

Analyzing the structure of IN-INI1 interaction should facilitate the functional studies. Because of the difficulties in purifying INI1 there is a lack of structural information. To overcome these difficulties, we have initiated *ab initio* computational homology-based modeling of INI1-IN interactions. Initial results indicate that there is extensive interaction between IN and INI1 and that some of the IN residues important for HIV-1 replication map to IN-INI1 interface. Our studies establish that binding of INI1 strongly influences HIV-1 replication and that further structural and functional analysis should lead to development of novel therapies to inhibit HIV-1 replication.

BACK

T72. Structural Basis of HIV-1 Vpu-Mediated BST2 Antagonism *via* Hijacking of the Clathrin Adaptor Protein Complex 1

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BST-2/tetherin is a potent antiviral restriction factor that protects against a broad range of enveloped viruses. It tethers the newly synthesized virions to the cell surface, preventing the viral release and spread. HIV-1 Vpu antagonizes BST-2 and overcomes BST-2 restriction through unclear mechanisms. In addition to the β-TrCP-mediated degradation pathway, Vpu causes the mistrafficking of BST-2, leading to the accumulation of newly synthesized and recycled BST-2 at the trans-Golgi network (TGN). However, the trafficking machinery involved has not been identified.

Here we present, together with biochemical and functional evidences, a crystal structure of the protein complex consisting of Vpu cytoplasmic domain (CD), BST2 CD and core of the clathrin adaptor protein complex 1 (AP1) to strongly suggest that HIV-1 Vpu hijacks AP1, thus the clathrin-dependent pathways, to mistraffick BST2. Our structural study was made possible by a rationally designed fusion chimera of BST2 CD and Vpu CD, which spatially mimics the arrangement of the two CDs that is dictated by the known transmembrane interaction between the two proteins. The structure reveals that Vpu modulates AP1 to greatly enhance its association with BST2. A novel open state of AP1 is observed, which is likely induced by Vpu. Given the phenotypical and functional resemblances, we further propose that hijacking the AP-dependent clathrin pathways might be similarly employed by Vpu to mistraffick and counteract other important immune factors. Thus, our study likely uncovers an evolutionarily conserved function of Vpu, which might be critical in understanding its full capacity in promoting the infectivity and pathogenesis of the primate lentiviruses.

BACK

T73. Structural Basis of Protein-Protein Interactions of Vpu from HIV-1

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Vpu is an 81-residue membrane protein encoded by HIV-1. It has two distinct domains, a hydrophobic transmembrane domain, which has been well studied (Park et al, 2006), and a cytoplasmic helical domain with two conserved phosphorylation sites, which has proven to be more difficult to study in large part because of its dynamics. Progress on the structure determination of the cytoplasmic domain and the full-length protein by NMR spectroscopy will be presented.

The base of knowledge about the transmembrane domain enables detailed studies of its interactions with two key host proteins to be investigated by NMR. Biochemical and spectroscopic studies show that Vpu employs a conserved mechanism to antagonize key surface proteins, in particular tetherin (BST-2) (Skasko et al, 2012) and NK, T-cell, B-cell antigen (NTB-A) (Sowrirajan et al, 2014). NMR spectral changes demonstrate that specific residues in Vpu's transmembrane domain interact with specific residues in the transmembrane domains of BST-2 and NTB-A. In combination with the emerging structural data on full-length Vpu, these interaction studies provide insights into the molecular mechanisms for Vpu's roles in overcoming cellular defense mechanisms.

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BACK

T74. Characterization of Vpu-SCF E3 Ligase Complex

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Viral protein U (Vpu) plays a critical role in HIV induced pathogenesis. It is the only transmembrane protein among all HIV accessory proteins and thus targets host membrane proteins. Vpu hijacks host SCF ubiquitin ligase complex (Cul1-Skp1-Rbx1-βTrCP) to ubiquitinate and downregulate the level of restriction factors including tetherin and CD4. Thereby it facilitates virus amplification and release. To understand how Vpu hijacks the SCF E3 ligase, we reconstituted and characterized the Vpu-E3 ligase protein complex that is consisted of Vpu cytoplasmic domain (VpuC), βTrCP-skp1, and Cul1-rbx1. Phosphorylated VpuC (pVpuC) forms a protein complex with purified βTrCP-skp1 and Cul1-rbx1. Phosphorylation of both Ser52 and Ser56 is essential for the formation of a stable pVpuC-βTrCP-skp1 complex, but phosphorylation of Ser52 is more critical than phosphorylation of Ser56 in forming the complex. We also reconstituted the full length Vpu (with transmembrane region)-E3 ligase protein complex, which is consistent with the idea that Vpu forms a homo-oligomer to carry out its function.

BACK

T75. How HIV-1 Nef Hijacks the AP-2 Clathrin Adaptor to Downregulate CD4

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The Nef protein of HIV-1 downregulates the cell surface receptor CD4 by hijacking the clathrin adaptor complex AP-2. The structural basis for the hijacking of AP-2 by Nef is revealed by a 2.9 Å crystal structure of Nef bound to the α and σ2 subunits of AP-2. Nef binds to AP-2 via its central loop (residues 149–179) and its core. The determinants for Nef binding include residues that directly contact AP-2 and others that stabilize the binding-competent conformation of the central loop. Residues involved in both direct and indirect interactions are required for the interaction of Nef with AP-2 and for downregulation of CD4. These results lead to a model for the docking of the full AP-2 tetramer to membranes as bound to Nef, such that the cytosolic tail of CD4 is situated to interact with its binding site on Nef.

BACK

F1. Antibody Affinity Maturation in an HIV Broadly Neutralizing B-cell Lineage: a Shift in Relative Orientation of Light- and Heavy-Chain Variable Domains

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Rapidly evolving pathogens, such as human immunodeficiency and influenza viruses, escape immune defenses provided by most vaccine-induced antibodies. Proposed strategies to elicit broadly neutralizing antibodies require a deeper understanding of antibody affinity maturation and evolution of the immune response to vaccination or infection. In HIV infected individuals, viruses and B-cells evolve together, creating a virus-antibody "arms race". Analysis of samples from an individual designated CH505 has illustrated the interplay between an antibody lineage, CH103, and autologous viruses at various time points. The CH103 antibodies, relatively broad in their neutralization spectrum, interact with the CD4 binding site of gp120, with a contact dominated by CDRH3. We show by analyzing structures of progenitor and intermediate antibodies and by correlating them with measurements of binding to various gp120s that there was a shift in the relative orientation of the light- and heavy-chain variable domains during evolution of the CH103 lineage. We further show that mutations leading to this conformational shift probably occurred in response to insertions in variable loop 5 (V5) of the HIV envelope. The shift displaced the tips of the light chain away from contact with V5, making room for the inserted residues, which had allowed escape from neutralization by the progenitor antibody. These results, which document the selective mechanism underlying this example of a virus-antibody arms race, illustrate the functional significance of affinity maturation by mutation outside the CDR surface of the antibody molecule.

BACK

F2. Investigations into the Lytic Mechanism of Peptide Triazole Thiols and the Comparison with HIV-1 Fusion

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Peptide triazoles (PTs) inhibit HIV-1 entry by binding to gp120, blocking gp120 interactions at both CD4 and co-receptor binding sites and causing gp120 shedding, consequently inactivating the virus irreversibly. PT-thiols typified by KR13 additionally cause lytic release of luminal p24 protein independent of cells [1]. This latter process was found to be related mechanistically to virus-cell fusion based on inhibition by fusion inhibitors such as T20 and 5-helix, and time-dependent exposure of the gp41 MPER epitope during lysis. Virolysis required both the gp120 binding pharmacophore (IXW) and the free thiol. Blocking the SH by capping or formation of obligatory dimers inhibited p24 release. To evaluate the role of the PT SH group, we investigated a series of PT-SH variants all containing IXW but with decreasing linker length to C-terminal SH. The peptides displayed a discontinuous relationship between virolysis and linker length, indicating that a minimal linker length is required for efficient virolysis. Inhibition of PT-SH induced virolysis by dithionitrobenzene, an active thiol blocking agent, as well as 2G12, an antibody that targets a disulfide-rich region on gp120, suggests that disulfide exchange between PT-SH and gp120 may be involved in the lysis process. Interestingly, disulfide exchange has been proposed to be important in the cell-virus fusion process [2]. Since lysis involves membrane perturbation, we examined the effects of methyl-b-cyclodextrin (MbCD) depletion of cholesterol, a major component of the virus membrane. We detected a bimodal response to MbCD in which KR13-induced lysis was enhanced at low concentrations of MbCD but completely lost at high concentrations. A similar bimodal response was observed in virus-cell infectivity with cholesterol depletion. While gp120 shedding correlates with loss of both KR13 lysis and cell infection of virus at high MbCD, the low MbCD effect may be more related to lipid bilayer perturbation. From the above results, we infer that the PT-SH induces a sequence of actions, in which binding to Env triggers gp120 shedding, disulfide exchange, conformational rearrangement of gp41 to a 6 helix bundle, MPER exposure and membrane transformation leading to virolysis. Deepening mechanistic understanding of the multi-step virolysis process may provide insights into the virus-cell fusion process itself and at the same time molecular tools for prevention and intervention of HIV-1 infection.

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BACK

F3. Potency Enhancement and Suppression of Infectious Virus Formation Using Multivalent Display of Peptide Triazole HIV-1 Entry Inhibitors on Gold Nanoparticles

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The peptide triazole family of HIV entry inhibitors binds to the Env surface protein gp120 and inhibits cell infection. Investigations to improve potency by exploiting multivalent effects were conducted by conjugating the cysteine-terminated peptide triazole KR13 to spherical gold nanoparticles *via* thiol linkage. The resulting AuNP-KR13 conjugate had close to 20-fold increased potency [1]. In addition, we surprisingly found that both the peptide triazole thiol and its nanoparticle conjugate were capable of disrupting the viral membrane, rendering the virus non-infectious. Though the disrupting activity of the peptide triazole thiol was theorized to derive from prematurely activating viral fusion mechanisms in the absence of a target cell [2], the exact mechanism and factors enhancing antiviral activity of the peptide triazole-nanoparticle conjugates are yet to be completely understood. In the present studies, both the diameter of the gold nanoparticles and the conjugation density of peptide triazoles were tested for their impact on antiviral potency. By increasing one or both of these properties of the nanoparticles, infection inhibition was found to reach picomolar IC_{50} values. At the same time, incorporating increasing proportions of a peptide triazole resistant gp120 mutant (S375W) on the virions led to decreasing infection inhibition and virus breakdown. These results suggest that the enhanced potency of peptide triazole-nanoparticle conjugates relies on the ability of the nanoparticles to encounter multiple Env spikes simultaneously. Enhanced potency against virions is not the only potential advantage engendered by nanoparticle conjugation. The protein gp120 is produced on the surface of infected cells as part of the viral life cycle. When treated with AuNP-KR13, transfected cells produced virions that exhibited impaired infectivity. At the same time, a precipitous drop in cell viability was observed at nanomolar AuNP-conjugated KR13 concentrations. We hypothesize that this cytotoxic activity is due to a similar multivalency effect as is responsible for the enhanced antiviral function, inducing sufficient strain to disrupt the cell membrane. Overall, the conjugation of peptide triazole to gold nanoparticles produces a multivalency-driven enhancement of peptide triazole's antiviral capabilities and further offers the possibility of using such multivalent conjugates to kill HIV Env-presenting cells. These findings provide a starting point to investigate an intriguing targeted therapy approach against both HIV virions as well as against their infected producer cells.

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BACK

F4. Functional Determinants of Peptide Triazole Dual Receptor Antagonists Utilize Two Conserved Hydrophobic Pockets of HIV-1 gp120

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We have developed a class of peptide triazole (PT) molecules that are able to inactivate HIV-1 virus by interacting with the envelope gp120 protein. This class of compounds inhibits gp120 interactions at both the CD4 and co-receptor binding sites. Determining the structural mechanism of PT interactions is being pursued to help define the mode of action of this class of virus inactivators and also to facilitate design of structure-minimized PT inhibitory molecules with improved stabilities and antagonistic functions. Site-specific mutagenesis and all-atom explicit solvent molecular dynamics (MD) had been employed previously[1] to investigate the putative functional site for peptide triazole inhibition on Env gp120. These analyses have identified binding pockets in gp120 for functionally important structural elements in PT's, and further have shown the capacity of PT's to stabilize an open conformation of gp120 containing an unstructured bridging sheet domain. We now have extended mutagenesis and applied model refinement using flexible docking analyses of PT-gp120 interactions. Our results show that PT can encounter gp120 utilizing two Env protein cavities. In our current model, the peptide Trp side chain interacts with well-conserved residues that form the CD4 Phe 43 binding pocket, namely Thr 257 and Ser 375. In addition, the aryl triazole moiety of PT was found to occupy a hydrophobic cavity gated by gp120 Trp112, a residue that is critical to PT-gp120 binding as well as to the folding of the gp120 bridging sheet used for co-receptor binding. We hope to use this evolving model, and eventually high-resolution structure analysis of core gp120 – minimized PT complexes that are currently being identified, to help guide design of peptidomimetic HIV-1 inactivators.

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BACK

F5. Structure-Based Design, Synthesis and Validation of Small Molecule CD4-Mimetic Inhibitors of HIV-1 Entry

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Entry of the human immunodeficiency virus (HIV) into human T-cells is typically initiated by a binding interaction between gp120, a component of the trimeric envelope glycoprotein spike on the virion surface, and CD4 on the host T-cell. The gp120-CD4 interface is dominated by a hydrophobic gp120 cavity capped by $Phe43_{CD4}$. Importantly, this hydrophobic cavity is a well-conserved feature among HIV-1 isolates, and as such comprises an excellent therapeutic target.

As part of a multidisciplinary program combining chemical synthesis with structural, thermodynamic, biochemical, virological and computational studies, we will present the design and evaluation of small molecules that disrupt HIV-1 entry by interacting with both the $Phe43_{CD4}$ hydrophobic cavity of gp120 and two additional binding "hotspots," displaying mid-nanomolar affinity and low-micromolar inhibitory concentrations. In particular, introduction of a functionalized indane scaffold permits suppression of CD4-independent viral entry observed with earlier generation compounds, and, importantly, sensitization of the virus to vaccine-elicited antibodies.

F6. Conformational Space Search and Analysis for Carbohydrates

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Carbohydrates and their derivatives, glycoconjugates, are well believed to play crucial roles in various biological processes, such as embryogenesis, signal transduction and immunity. But their functions and dynamics during those processes are not fully understood. In addition, saccharides recognized by other glycans, enzymes or lectins were found to prefer certain folded structures. Therefore in order to improve our understanding of glycans, accurate prediction of their structures is required. Unfortunately, available experimental data is far from enough and interpretations about them have no uniform criteria and bear intrinsic drawbacks. However, using molecular mechanism methods with libraries of peptides rotamers, we are able to generate enough meaningful data and gain insights into structures and functions of proteins and nucleic acids. Hence, to set up libraries for glycans, our approach is to get detailed maps of the free energy landscape for all the biologically important disaccharides and then extract local minima as initial structures for building oligosaccharide models. Instead of molecular dynamics (MD) simulations which would only visit certain conformations during a typical MD run, we use Monte Carlo (MC) simulation to explore the whole conformational space.

Our preliminary results show clear convergence patterns for glycosidic linkages.

F7. Understanding Glycan Type Specificity in Highly Glycosylated Proteins

Artem Krantsevich and David F. Green

Stony Brook University

Despite the fact that the vast majority of glycans in most known glycoproteins are of complex type, some glycoproteins have a clear tendency to contain high-mannose and hybrid structures as well. For example, HIV envelope glycoprotein gp120 usually has about 24 glycans, and almost half of them are either high-mannose or hybrid. Statistical analysis of gp120 suggests that: 1) glycosylation sites placed closer to the C-terminus of the protein tend to carry high-mannose/hybrid glycans; 2) glycosylation sites placed in flexible loops tend to carry complex glycans; and 3) glycosylation sites placed in regions of secondary structure tend to carry high-mannose/hybrid glycans. Analysis of glycosylation sites placed in other fucose-containing glycoproteins' secondary structure was inconclusive, though our results for highly glycosylated proteins were consistent with our hypothesis. In order to develop a mechanistic understanding of the origin of these differences, we developed a stochastic chemical-kinetic model of the N-linked glycosylation pathway. This model provides some understanding of how the distribution of glycan types depends on: 1) concentration of glycans; and 2) parameters that describe certain glycan modifications.

BACK

F8. Statistical Analysis of the Glycosylation Sites in HIV-1 Envelope Protein

Nikolay Krantsevich and David F. Green

Stony Brook University

Envelope glycoprotein gp-120 plays a key role in the life cycle of the HIV viral cells; gp-120 is a highly-glycosylated protein which carries 20-25 glycans that are important for its proper functioning. In this study, we have concentrated on the statistical analysis of the amino acid sequences of the HIV-1 envelope protein with respect to glycosylation sites. The goal is to get some insight on glycosylation site positioning along the protein sequence and to identify certain patterns if any exist. Conservation of glycosylation sites in both conserved and variable regions of the protein sequence were considered. Pairwise site analysis for conserved regions did not yield any evident correlation between any two sites, although the set of highly conserved positions was identified. The results obtained for variable regions show that there are some certain glycosylation patterns preserved, although differences in loop lengths and low level of conservation make it difficult to characterize those patterns. In order to resolve this problem, we analyzed the distributions of glycosylation site number and distributions of distances between glycosylation sites for the subtypes of HIV1 group M; the obtained results provided some confirmation to the existence of glycosylation patterns in variable regions.

BACK

F9. Understanding Dynamic Structural Variations in HIV-1 Envelope Glycoprotein gp120

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HIV-1 envelope glycoprotein gp120 subunits, together with transmembrane glycoprotein gp41, form trimeric spikes on the viral surface. These spikes play a crucial role in initiating HIV-1 infection by binding of gp120 to CD4 receptors and subsequently to coreceptors (CCR5 or CXCR4) on target cells. A full investigation of gp120 dynamics within and between different functional states is of importance to understand protein function and guide vaccine design. To this end, we have performed three independent long-time (1 μs+) molecular dynamics (MD) simulations for the unglycosylated monomeric gp120 core in its free, CD4-bound, and antibody-bound states. Convergence of each trajectory was measured and discussed in quasiharmonic (principal component analysis (PCA)) and clustering analysis. Upon verifying the reliability of our simulations, we studied the dynamics of bound and unbound gp120 extensively by calculating pairwise RMSD of domains, evolution of secondary structure per residue, collective and global motions of gp120, fluctuations of residues, and characteristic configurational ensembles and their transitions. We found that unbound gp120, though having much slower and more correlated modes of motions among domains than bound ones, showed a very good conservation of secondary structure, tertiary structure and dynamics in the 1.6 μs trajectory. Long distance coupled motions and structural changing patterns of unbound gp120 were described in detail and their differences from that of the bound ones were highlighted.

BACK

F10. Conformational Dynamics Of Single HIV-1 Env Trimers On The Surface Of Native Virions

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The HIV-1 envelope (Env) mediates viral entry into host cells. While static images of Env define distinct conformations, direct observations of Env dynamics have yet to be realized. To enable real-time imaging of conformational events in Env, we introduced fluorophores into the variable regions of the Env gp120 subunit and measured single-molecule fluorescence resonance energy transfer (smFRET) within the context of native trimers on the surface of HIV-1 virions. We observed unliganded HIV-1 Env to be intrinsically dynamic, transitioning between three distinct pre-fusion conformations, and receptor CD4 and co-receptor surrogate antibody 17b to remodel the conformational landscape of Env. Differences in conformational dynamics and ligand responsiveness of neutralization-sensitive and neutralization-resistant HIV-1 isolates delineated the dynamics-based mechanism of immune evasion. smFRET also revealed neutralizing antibodies VRC01, PG16, PGT128, PGT145, and 2G12, and the entry inhibitor, BMS-626529, to stabilize the ground state of Env, thereby providing dynamics-based strategies for therapeutic intervention.

BACK

F11. The Structural Stability of gp120 and Trimeric gp140

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The structural stability of the HIV-1 YU2 glycoprotein gp120 and trimeric gp140 has been studied as a function of pH by differential scanning calorimetry and isothermal chemical denaturation. The gp140 used in the studies is uncleaved and contains the ectodomain of gp41 fused with a fibritin trimerization motif. Both gp120 and gp140 were expressed in human 293 F cells and are fully glycosylated. A thermal scan of gp120 at pH 7.5 shows a single transition centered at 61°C and the shape and the area of the peak agrees with two-state unfolding. Scans of gp140 shows two unfolding transitions, one centered at 61°C, the other at 84.5°C at pH 7.5. The transition in gp140 that occurs at the lower temperature can be described by a monomeric cooperative unit of equal enthalpy to that of gp120, suggesting that the gp120 subunits within the trimer do not interact strongly and unfold independently of each other. The shape and the area of the second unfolding transition are characterized by a cooperative unit close to three indicative of the unfolding of highly interacting structural elements within the trimer. The results are corroborated by urea denaturation studies, which show that gp120 has a single transition whereas the denaturation of gp140 is described by a three-state model.

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F12. Maximum-Unbiased Benchmarking Datasets for Ligand-Based Drug Discovery targeting Human Chemokine Receptorome

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Human chemokine receptors are members of G protein-coupled receptors (GPCRs) superfamily, one of the most exploited drug targets resulting in the discovery of over 30-40% of US marketed therapeutics. Human chemokine receptorome includes CXC, CC, C and CX3C receptor subfamilies. All of them play a variety of key roles in the regulation of a plethora of physiological processes such as cognition, metabolism, inflammation, immunity and cell proliferation. Thus, chemokine receptors represent a multitude of attractive targets for modern drug discovery. However, crystal structures for most members of this superfamily have not been solved up to now, which significantly restricts structure-based drug discovery. In this way, ligand-based drug discovery (LBDD), another important strategy for early-stage drug discovery, becomes more instrumental and practical to employ. The LBDD includes a variety of methods, such as similarity search, pharmacophore modeling and quantitative structure–activity relationship (QSAR) modeling. Normally, the retrospective small-scale virtual screening based on public or self-built benchmarking sets is conducted to select the optimum screening method(s) prior to the large-scale screening efforts as part of the routine for modern drug discovery. However, the lack of standard decoy datasets for chemokine receptorome introduces bias into this benchmarking process.

Recently, we developed an unbiased method to build benchmarking sets for ligand-based virtual screening (LBVS). Herein, we applied this method to build benchmarking sets for chemokine receptorome. Starting from collecting the biologically active, drug-like from the ChEMBL database, we screened ZINC database to select suitable decoys for each subtype of chemokine receptors. The quality of the benchmarking sets was validated by the exhaustive Leave-One-Out (LOO) Cross-Validation (CV). Results show good quality of the benchmarking sets, in which the ROC AUCs are close to 0.5 and curves are close to random distribution. The property distributions within the decoy sets also match well to their respective ligand sets. We anticipate the benchmarking sets will be substantially useful for our ongoing ligand-based drug discovery efforts for CXCR4/CCR2 Dual-targeted Fusion Inhibitors.

BACK

F13. The Dependence of HIV Env-Mediated Fusion on Phosphatidylserine in the Target Cell Membrane

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Like other enveloped viruses, HIV-1 delivers its RNA into cells by fusing the viral envelope with the cell membrane. This fusion process is driven by conformational changes in the HIV envelope protein (Env), which are triggered by Env interactions with CD4 and cytokine coreceptors. Our results substantiate the hypothesis that this fusion process also depends on interactions between Env and phosphatidylserine (PS), in the cell membrane. Although PS, the most abundant anionic lipid in mammalian membranes, is found mostly in the inner leaflet of the plasma membrane, it is transiently translocated to the outer membrane leaflet in many cell activation pathways. Our data indicate that Env-co-receptor interactions induce PS externalization dependent on intracellular calcium signaling and mediated by TMEM16F, a putative phospholipid scramblase. Externalized PS promotes Env-mediated fusion downstream of gp120-coreceptor binding and upstream of interaction between gp41 and the target membrane, and hemifusion. Suppressing TMEM16F scramblase activity inhibits single-round infection by HIV pseudoviruses. Finding that CD4- and coreceptor-dependent HIV fusion also depends on externalized PS suggests an intriguing interplay between infection and the activation status of the invaded cell.

BACK

F14. Small Molecule Docking Using Property-Based Volume Overlap

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Ongoing work in our laboratory has explored use of property-based volume overlap as a new scoring function for DOCK. The goal is to aid identification of lead compounds occupying similar space in a binding site, and having matched chemical properties, as a known reference ligand. Our initial studies employed a grid-based overlap calculation method which worked well for rescoring, but was prohibitively slow for on-the-fly sampling. To address issues with speed, we are testing a fast volume overlap approximation method recently reported by Sastry et al. (*J. Chem. Inf. Model.* **2011**, *51*, 2455). Progress towards validation and application of a volume overlap scoring function by itself or in combination with other DOCK scoring functions for virtual screening and *de novo* design, including the HIVgp41 system being targeted in our laboratory, will be discussed.

BACK

F15. Human Neutrophil Peptide 1 Sensitizes HIV-1 to Antibodies and Inhibitors Targeting Intermediate Conformations of gp41

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Human α-defensins inhibit HIV-1 infection in serum-free media, but their activity is strongly attenuated by serum. We examined the effect of human α-defensin HNP-1 on early steps of virus entry/fusion in the presence of serum. In spite of the modest effect on the extent of fusion, HNP-1 prolonged the exposure of the gp41 pre-hairpin intermediates on the cell surface by delaying the post-coreceptor binding steps of HIV-1 entry. The prolonged lifetime of pre-hairpin intermediates correlated with the marked sensitization of HIV-1 to neutralizing anti-gp41, but not anti-gp120 antibodies. The most prominent potentiating effect of α-defensin was on antibodies and peptides targeting the first heptad repeat domain of gp41. At sub-inhibitory concentrations, HNP-1 also promoted the inhibition of virus entry into peripheral blood mononuclear cells by anti-gp41 antibodies and HIV-1 immune serum. Our findings thus imply that the efficiency of fusion inhibitors and neutralizing antibodies targeting the first heptad repeat domain of gp41 is kinetically restricted. These results suggest new strategies to render HIV-1 vulnerable to antibodies and inhibitors binding to the first heptad repeat domain of gp41 through delaying the clearance of pre-hairpin intermediates from the cell surface. This work was supported by the R21 AI087453 and R01 GM054787 grants.

BACK

F16. gp41 Ectodomain Dissociates and Forms a Stable Monomer on Phospholipid Vesicles and Detergent Micelles: Implication for the HIV-1 Env-Mediated Membrane Fusion

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The first step of HIV infection involves the fusion of the viral and target cell membranes, a process mediated by the viral envelope glycoproteins, gp120 and gp41. The binding of gp120 to the cell surface receptors CD4 triggers a cascade of conformational changes that disrupt the gp41-gp120 interactions and allows the insertion of the N-terminal fusion peptide of gp41 into the host cell membrane, while the host cell and viral membranes are separated by the length of the extended gp41 ectodomain. After fusion is complete, the gp41 trimer is known to have collapsed into a highly stable 6-helix bundle (6HB) conformation, with close spatial proximity between its fusion peptides and transmembrane domains, originally anchored in the host cell and viral membranes, respectively. Notwithstanding its critical importance for the design of fusion inhibitors, very little structural information is known about the gp41 transition from its initial extended pre-fusion to its compact post-fusion 6HB state. To investigate the dynamic and structural properties of intermediate metastable states, we designed a set of protein constructs mimicking the extra-cellular ectodomain of gp41. We found that the structure of the gp41 ectodomain is pronouncedly impacted by the presence of phospholipid vesicles or detergents containing phosphatidyl choline head groups and can switch between a trimeric 6HB state and a monomeric membrane bound state. The structure of the gp41 ectodomain in a monomeric state was determined by state-of-the-art NMR techniques, including measurements of NOE distance restraints and residual dipolar couplings in weakly aligned solutions. ^{15}N relaxation data provided a detailed view at the backbone dynamics of both the trimeric and monomeric conformations. The monomeric state is postulated to represent the pivotal structural intermediate enabling the gp41 conformational change during the fusion process and responsible for driving the local apposition of the viral and cellular membranes required for fusion.

F17. HIV Resistance Mechanisms to D-Peptide Entry Inhibitors

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Our lab has developed a potent D-peptide inhibitor of HIV entry, PIE12-trimer, which targets the highly conserved gp41 N-trimer ‘pocket’ region. This inhibitor is highly resistant to proteolysis and broadly inhibits all major clades with high pM to low nM potency. Conjugation of PIE12-trimer localizes it to cell membranes and improves potency by more than 100-fold. Cholesterol-conjugated PIE12-trimer is in preclinical studies for HIV prevention and treatment. Although PIE12-trimer has a high barrier to resistance (‘resistance capacitor’), resistant virus can be generated via extended passaging in the presence of slowly escalating inhibitor. Here we analyze the mechanism of this resistance using deep sequencing, crystallography, binding studies, and viral fitness measurements. This information will help predict likely drug resistance pathways and their associated fitness penalties and inform the design of next generation inhibitors.

F18. Pharmacophore Matching Similarity Score In DOCK With Application To HIVgp41

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In this study, a 3D pharmacophore matching similarity (FMS) scoring function has been implemented into the structure-based design program DOCK and validated using pose reproduction on a small molecule database of 1043 systems, as well as tested in crossdocking and enrichment experiments. Compared to the standard DOCK single grid energy (SGE) score, FMS score yields ~10% increase in docking success and ~6% drop in sampling failure. And when used in combination, FMS+SGE score further boosts the success rate to 98.3%. Importantly, the scoring and sampling failure rates are low when FMS is employed. Overall, FMS+SGE score yields the highest matrix success rates when tested on six crossdocking families. In cases where the reference binding poses across the systems are reasonably well overlapped in pharmacophore space, good crossdocking success rates are obtained. FMS and FMS+SGE scores have also been tested using DUD-E enrichment studies, and again in most cases result in improved enrichment over SGE score. Progress towards employing FMS/FMS+SGE score in de novo design using DOCK will be presented as well as virtual screening targeting HIVgp41.

F19. Reverse Hairpin Constructs of the gp41 Ectodomain are Potent Fusion Inhibitors by Virtue of an Exposed Hydrophobic Pocket on the N-Heptad Repeat

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HIV-1 glycoprotein-41 (gp41) mediates membrane fusion, enabling entry of HIV-1 virus into host cells. It undergoes conformational rearrangement after viral attachment, resulting in a trimer of hairpins (TOH) post-fusion structure. Long-lived prehairpin intermediates (PHI) that are vulnerable to inhibition contain exposed N-heptad repeat (NHR) and C-heptad repeat (CHR) domains and are the target of fusion inhibitors. We have designed reverse hairpin gp41 ectodomain mimics representative of parts of the PHI and TOH, and which expose NHR domain to differing degrees. Despite having no artificial stabilization of the NHR trimer, they are potent nM inhibitors of fusion provided that the hydrophobic pocket and an extended C-terminal groove on the NHR are exposed. We performed biophysical and biological experiments on the proteins to establish their properties and mechanism of action. Data point to a different mechanism than that of CHR peptides, implying a different resistance profile and suggesting important properties of the prehairpin intermediates. In addition, the constructs contain two immunosuppressive motifs, and a third is planned.

BACK

F20. Computer-Aided Design of Small Molecule Inhibitors of HIVgp41

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Despite the challenges associated with developing small molecule inhibitors that target HIVgp41, development of potent low-molecular weight compounds would be a promising alternative to peptide-based drugs such as T20 (Fuzeon). Building upon a successful virtual screen performed previously in our laboratory (*Bioorg. Med. Chem. Lett.* **2012**, *22*, 3011-3016), we are using computer-aided design techniques in an attempt to optimize the activity of several small molecule leads with verified experimental activity. All-atom de novo design, re-docking, and molecular dynamics methods are being used in conjunction with binding energy estimations, footprint stability, and pose stability to prioritize compounds for possible synthesis. Examples of promising in silico analogs, based on parent compounds, which were modified through R-group substitution and ring cyclization will be presented.

BACK

F21. Small Molecule Inhibition of HIV gp41 NHR Trimer Formation

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HIV-cell fusion inhibition by peptides, antibodies, or small molecules targeting the viral fusion protein gp41 is an active area of research. Peptides and small molecules that bind the N-terminal heptad repeat (NHR) trimer, thus obstructing 6-helix bundle formation, have been shown to quantitatively inhibit virus cell fusion, although only one peptide has been approved by the FDA: T20 (Fuzeon). A possible alternative mechanism for inhibiting virus-cell fusion is to employ peptides based on the NHR sequence (e.g. N36) to prevent NHR trimer formation. Such peptides can prevent fusion but do not have the appropriate pharmacokinetics or pharmacodynamics for clinical use. We hypothesize that small molecules which mimic the activity and mechanism of N36 may avoid the pitfalls of peptide-based inhibitors but still inhibit NHR trimer formation, thus preventing virus-cell fusion. To that end, we performed molecular dynamics simulations of the NHR trimer, followed by detailed surface curvature analyses to identify two potential "hot-spots" for small molecules at the interface of the three helices. We virtually screened a library of 1.4 M compounds with the program DOCK6 to each of the two putative pockets, and 120 compounds were purchased for experimental testing. In a cell-cell fusion assays, four of those compounds exhibited dose-dependent anti-fusion at the low micro-molar level and relatively low levels of cytotoxicity. Experiments to provide additional biochemical evidence for the specific inhibition mechanism are planned.

F22. The Tripartite Motif Coiled-Coil Is an Elongated Antiparallel Hairpin Dimer

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Tripartite motif (TRIM) proteins make up a large family of coiled-coil-containing RING E3 ligases that function in many cellular processes, particularly innate antiviral response pathways. Both dimerization and higher-order assembly are important elements of TRIM protein function, but the atomic details of TRIM tertiary and quaternary structure have not been fully understood. Here, we present crystallographic and biochemical analyses of the TRIM coiled-coil and show that TRIM proteins dimerize by forming interdigitating antiparallel helical hairpins that position the N-terminal catalytic RING domains at opposite ends of the dimer and the C-terminal substrate-binding domains at the center. The dimer core comprises an antiparallel coiled-coil with a distinctive, symmetric pattern of flanking heptad and central hendecad repeats that appear to be conserved across the entire TRIM family. Our studies reveal how the coiled-coil organizes TRIM25 to polyubiquitylate the RIG-I/viral RNA recognition complex and how dimers of the TRIM5α protein are arranged within hexagonal arrays that recognize the HIV-1 capsid lattice and restrict retroviral replication.

BACK

F23. Hydrogen/Deuterium Exchange Studies of TRIM Family Proteins

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The TRIM family proteins, TRIM 5a and TRIM-Cyp act as retroviral restriction factors. Structurally they are composed of RING, B-box, and coiled-coil domains as well as either a SPRY domain (TRIM 5 a) or a cyclophilin domain (TRIM-Cyp). These latter domains are responsible for recognizing HIV capsid protein. Recent crystallographic and biochemical analysis has demonstrated that the coiled-coil domain is anti-parallel and suggested that the SPRY domain is docked against a platform formed by the coil-coil (1).

In support of this model, H/D exchange studies of rhesus derived TRIM 5 a CC-SPRY in comparison to the isolated SPRY domain have identified regions of the SPRY domain that are protected in CC-SPRY and thus are candidates for the docking site. Chemical crosslinking studies are underway to obtain distance constraints that will prove useful in modelling the docking arrangement.

To explore the binding of CC-Cyp to HIV CA, CC-Cyp was bound to disulfide stabilized hexamers and the protection of the bound hexamers compared to hexamers free in solution. Protection of CTD helix 9 was observed in the complex at the earliest time points. The H/D exchange pattern was bimodal indicating that a subset of the hexamers were exchange protected. This data suggests that the cyclophilin domains are positioned in such as way as to bring two hexamers into sufficient proximity to allow CTD dimerization to occur.

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Reference:

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BACK

F24. The Role of the SPRY Domain V1 Loop Flexibility in the Restriction Activity of TRIM5alpha.

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The mobile V1 loop within the C-terminal SPRY domain is a key determinant of capsid specificity in the TRIM5alpha-mediated restriction of retroviral infection. For example, the rhesus monkey TRIM5alpha potently restricts HIV infection, whereas the human variant of this restriction factor is not active against HIV. Mutagenesis studies revealed that mutations within the V1 loop can restore HIV restriction by the human TRIM5alpha, with the R332G/R335G double mutant approaching the potency of the rhesus variant. We use replica exchange molecular dynamics (REMD) to investigate the V1 conformations of the rhesus (rhWT), human wild-type (huWT) and human R332G/R335G double mutant (huDM) SPRY domains. The V1 loops in the rhWT and huDM SPRY variants exhibit multiple divergent conformations in agreement with high intrinsic disorder revealed by the NMR studies. In contrast, dynamics of the huWT V1 loop is more restrained and can be described as a fluctuation in the vicinity of a single structured state. The apparent correlation between the flexibility of the V1 loop and the restriction activity of a particular SPRY variant is further supported by the REMD studies of the Y336K mutant of the human SPRY, in which substantial flexibility of the V1 loop was also observed. The mechanistic links between V1 conformations and restriction are explored by docking of the REMD-derived SPRY conformations onto the assembled structures of several viral capsid variants.

BACK

F25. Alpha-Helices in the Coiled-Coil Linker Region Govern Rhesus TRIM5α Assembly and Restriction

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The rhesus TRIM5 alpha (rhTRIM5α) belongs to the tripartite motif (TRIM) containing ubiquitin E3 ligase family and consists of RING, B-box, coiled-coil (CC) and B30.2 (SPRY) domains. *In vitro* studies have shown that dimeric rhTRIM5α assembles to form hexagonal sheet-like structure. The higher order structure is thought to embrace retrovirus capsid and interacts with it via multiple C-terminal SPRY domains. Cytoplasmic assemblies of rhTRIM5α are manifested as punctate bodies. However, the exact mechanism of rhTRIM5α assembly as well as its role in HIV restriction is not yet clearly understood. Our observations suggest that the alpha-helices present in the Linker 2 region, which connects the coiled-coil domain to SPRY domain, plays key role in the formation of cytoplasmic bodies as well as virus restriction. Using mutagenesis of the Linker 2 region, we observe that the amount of helical content in the region is directly proportional to the amount of cytoplasmic body formation as well as degree of virus restriction. Fluorescence Resonance energy transfer (FRET) analysis indicates that the CC-L2 dimer exists as an antiparallel dimer in cells, consistent with a recent report. Moreover, using the recently published structure of the TRIM25 CC-L2 dimer, we have generated a homology model of the rhTRIM5α CC-L2 dimer. We find that mutations that disrupt assembly and restriction reside in α-helical contacts which interact with helices of the CC region. Molecular simulations recapitulate our *in vivo* observations, as these helices dock normally in the wt CC-L2 dimer but fail to down when mutations are introduced which disrupt assembly and restriction. We are presently utilizing single molecule FRET analysis of the CC-L2 dimer to determine how mutations in the L2 region affect the proper docking of the L2 helices to their cognate CC regions. These studies will define how residues in the L2 region promote docking of the L2 helices to CC helices during assembly, providing molecular insight into the formation of TRIM5α assemblies during restriction.

F26. Electron Microscopic and Biochemical Characterization of Recombinant TRIM5α Proteins and Their Capsid Complexes

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The mammalian restriction factor TRIM5α inhibits retroviral replication by binding to viral capsids, accelerating their dissociation, and blocking reverse transcription of the viral genome (1, 2). Avid binding to capsids requires TRIM5α self-assembly into higher order structures. We previously showed that a non-native restricting chimeric TRIM5 construct, termed TRIM5-21R, can assemble into hexagonal arrays *in vitro* and that TRIM5-21R assembly is stimulated by HIV-1 capsid surface mimics (3). We therefore proposed a model in which TRIM5α recognizes retroviral capsid surfaces by forming a complementary hexagonal lattice. However, direct experiments to define how TRIM5α binds viral capsids have not yet been performed using native TRIM5α proteins and HIV-1 cores.

Here we report the expression and purification of multimilligram quantities of native TRIM5α proteins from several different primates. Purified recombinant TRIM5α proteins were dimers as determined by analytical ultracentrifugation, and could assemble into hexagonal arrays, demonstrating that hexagonal lattice formation is conserved across different TRIM5α alleles. We also designed and isolated hyperstable, disulfide-crosslinked HIV-1 cores suitable for biochemical and electron microscopic studies, using a novel affinity purification strategy. Electron cryo-tomography images revealed that the capsid lattice contained hexagonal and pentagonal subunit assemblies, as predicted by the fullerene cone model. Pure recombinant TRIM5α proteins bound directly and specifically to hyperstable capsids *in vitro,* and binding correlated well with the ability of different TRIM5α alleles to restrict HIV-1. EM imaging studies revealed that TRIM5α forms a deformable hexagonal net on the capsid surface, and the interhexameric spacings (~35 nm) agreed well with the 2D crystal lattices formed by TRIM5α. We therefore conclude that TRIM5α recognizes retroviral capsids by assembling highly deformable hexagonal nets that can accommodate the varying curvature and morphologies of retroviral capsids.

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BACK

F27. Correlated Cryogenic Light and Electron Microscopy to Visualize Cytoplasmic TRIM5α Bodies

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The mammalian TRIM5α protein is a host restriction factor that inhibits retroviral replication in a species-dependent manner. HIV-1 restriction by rhesus (rh) TRIM5α prevents the viral pre-integration complex from entering the nucleus by binding and targeting the HIV-1 capsid at multiple stages in the cytoplasm. The exact mechanism by which rh-TRIM5α restriction occurs is uncertain, but *in vitro* studies have previously revealed this is likely to occur through TRIM5α recognition of retroviral capsid surfaces by forming a complementary hexagonal lattice (1,2,3). We have produced the first electron cryo-tomography (ECT) images of recombinant TRIM5α forming deformable hexagonal nets on isolated hyperstable, disulfide-crosslinked HIV-1 cores.

Within cells, TRIM5α forms dynamic cytoplasmic bodies (4). However, their *in vivo* structure and potential role in TRIM5α function have yet to be determined. Additionally, we also present methods outlining the use of correlated cryogenic light microcopy (LM) and electron microscopy (EM) to visualize cytoplasmic TRIM5α bodies in HELA cells. HELA cells stably expressing TRIM5α-YFP were grown and plunge frozen with red fluorescent beads on gold finder EM grids. The TRIM5α-YFP signal detected by fluorescent LM was correlated using the fluorescent beads and imaged at higher magnification using ECT. In conclusion, this work will ascertain the structural nature of HIV-1 restriction by rh-TRIM5α *in vivo.*

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BACK

F28. Another Connection Between Capsid and Reverse Transcription

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A picture is emerging that reveals important connections between reverse transcription and uncoating. For example, previous work has revealed that inhibiting reverse transcription can cause a delay in uncoating. To gain further insights, we utilized an HIV derivative that contains 2 cysteine substitutions, which cause the formation of a hyperstable capsid structure (Pornillos et al, Cell. 2009; 137(7):1282-92). Viruses containing these changes are noninfectious. We then began to compare the hyperstable and wt HIV in imaging studies. We followed the fate of HIV cores by the use of the immunofluorescence super-resolution technique 3D-SIM, as well as live cell imaging using wide-field microscopy and deconvolution. In order to study uncoating, we used the HIV-iGFP construct, into which GFP is translated within the interspatial cleavage sites of MA and CA of both WT and CC1 HIV viruses. These viral particles are able to trap GFP inside of mature viral cores that are released into the cytoplasm after fusion. The trapping of GFP inside of the viral core has been previously shown by the use of gradient ultra-centrifugations of membrane stripped viruses as well as by using a TRIM5a capsid integrity assay that captures HIV capsids in the presence of the drug MG132. Labeling with iGFP and mCherry-Vpr allows the state of integrity of the capsid to be elucidated. Attempts to label reverse transcription of the CC1 virus generated negative results. Further analysis revealed that the CC1 mutant viruses are not able to perform any detectable reverse transcription. No early products of RT can be detected. However, lysing the virions revealed an active RT using in vitro reverse transcription assays. Most remarkably, we were partially able to restore HIV CC1 infection by a reduction treatment with DTT or DTBA prior to cell challenge and measuring infection titers by the use of Flow Cytometry, thus showing that the RT process is highly interconnected, if not dependent, on the state of the capsid core of the virus. The block of reverse transcription by disulfide bonds which stabilize the capsid core structure reveals the connection between the state of the capsid and reverse transcription and also reveals a new level of the regulation of the early steps of HIV replication

BACK

F29. A Structure-Based Mechanism for tRNA and Retroviral RNA Remodeling During Primer Annealing

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In order to prime reverse transcription, retroviruses require annealing of a tRNA molecule to the U5-primer binding site (U5-PBS) region of the viral genome[1,2]. The residues essential for primer annealing are initially locked in intramolecular interactions[3-5], and hence, annealing requires the chaperone activity of the retroviral nucleocapsid (NC) protein to facilitate structural rearrangements[6]. Here we show that, unlike classical chaperones, the Moloney murine leukemia virus NC uses a unique mechanism for remodeling: it specifically targets multiple structured regions in both the U5-PBS and $tRNA^{Pro}$ primer that otherwise sequester residues necessary for annealing. This high-specificity and high-affinity binding by NC consequently liberates these sequestered residues—which are exactly complementary—for intermolecular interactions. Furthermore, NC utilizes a step-wise, entropy-driven mechanism to trigger both residue-specific destabilization and residue-specific release. Our structures of NC bound to U5-PBS and $tRNA^{Pro}$ reveal the structure-based mechanism for retroviral primer annealing and provide insights as to how ATP-independent chaperones can target specific RNAs amidst the cellular milieu of non-target RNAs.

BACK

F30. SAXS Analysis Reveals Conservation of tRNA Structural Mimicry in the HIV-1 5´UTR and Model of the RT Initiation Complex

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Human lysyl-tRNA synthetase (LysRS) and $tRNA^{Lys}$ isoacceptors are specifically recruited into HIV-1 particles where $tRNA^{Lys3}$ serves as the primer for reverse transcription. The mechanism by which $tRNA^{Lys3}$ is recruited into HIV-1 is incompletely understood. We have recently shown that LysRS binds to a U-rich loop in the 5´UTR region of the viral RNA genome immediately upstream of the primer-binding site (PBS). This interaction competitively displaces $tRNA^{Lys3}$ from LysRS, providing a plausible mechanism for primer release from LysRS and targeting to the PBS (1). This U-rich loop is a tRNA-like element (TLE) that mimics the $tRNA^{Lys}$ anticodon loop, a critical recognition element for LysRS. Small-angle X-ray scattering (SAXS) analysis of a 105-nt RNA (PBS_{105}) containing the PBS and TLE of the NL4-3 5´UTR, both in the absence and presence of an annealed $antiPBS_{18}$ DNA oligonucleotide, revealed that the region further mimics tRNA by adopting a similar overall shape (2). Consistent with this result, the affinity of LysRS for PBS_{105}:$antiPBS_{18}$ is similar to that for $apoPBS_{105}$. LysRS interacts with similar affinity to PBS_{105}:primer complexes containing full-length and 3'-half $tRNA^{Lys3}$ constructs. Annealing of primers with additional 3´ deoxynucleotides designed to mimic reverse transcriptase (RT) extension products, reveals that LysRS binding affinity is reduced upon primer extension into the TLE stem. We also examined HIV-1 RT binding to these same PBS:primer complexes and generated a preliminary structural model of the PBS_{105}:$antiPBS_{18}$:RT reverse transcription initiation complex (RTIC).

While NL4-3 (representative of clade B) is one of the most studied HIV-1 isolates, another frequently examined isolate is MAL (representative of clade A in its 5´UTR). MAL contains a 23-nt insertion in the 5´UTR, resulting in a significantly different predicted secondary structure obtained by chemical probing (3). We show that the affinity of LysRS for the NL4-3 and MAL PBS domains is similar and SAXS analysis reveals that the two viral isolates share a strikingly similar overall shape. Thus, tRNA structural mimicry in the 5´UTR is a conserved feature of distinct viral subtypes, suggesting a common mechanism of tRNA primer recruitment and RTIC formation.

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BACK

F31. RNA Structural Regulation of Reverse Transcription Initiation in HIV

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The initiation of reverse transcription in HIV is the first step of infection after the virus enters the host cell. During the reverse transcription process the single-stranded RNA genome of the virus is reverse transcribed by the enzyme reverse transcriptase into circular, double-stranded DNA, which subsequently integrates into the host cell genome. Reverse transcription initiation occurs at the 5' end of the viral genome where a prepackaged host-cell $tRNA^{Lys}{}_3$ primer is annealed. The 3' -OH of the tRNA serves for the site of initiation. Extensive work has shown that this initiation complex is highly structured and that mutation of these RNA structures affects initiation kinetics and virus viability (Isel *et al.* 1993, Isel *et al.* 1998, Liang *et al.* 1998). Using NMR spectroscopy, we show that the initiation complex is heterogeneous in nature. smFRET spectroscopy allows us to monitor the conformation of individual molecules from these heterogeneous complexes and shows that reverse transcriptase has the ability to rearrange the initiation complex's structure. By combining these real-time techniques with X-ray crystallography, we seek to explain how RNA conformations regulate reverse transcription initiation in HIV.

BACK

F32. Ty3 Reverse Transcriptase Complexed with an RNA-DNA Hybrid Shows Structural and Functional Asymmetry

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Retrotransposons are mobile genetic elements that replicate through an RNA intermediate. They are divided into two groups, depending on the presence of flanking long-terminal repeat (LTR) sequences. Approximately 40% of the human genome is derived from retroelements with 8% corresponding to the LTR class. Retroviruses, such as human immunodeficiency virus (HIV-1) evolved from LTR elements through acquiring the envelope genes which allow them to leave the cell and infect other cells. Reverse transcriptase (RT) is a critical enzyme of retroelements, combining DNA polymerase and RNase H activities to convert the (+) strand RNA genome into double-stranded DNA that is competent for integration. However, In contrast to the extensive structural characterization of retroviral RTs, detailed information on the enzymes from LTR-containing retrotransposons such as Ty3 of *Saccharomyces cerevisiae,* is lacking.

Ty3 belongs to the Gypsy family and its RT is perhaps the most extensively characterized LTR-retrotransposon enzyme with respect to its catalytic activities, as well as the nucleic acid duplexes with which it interacts. Although structural motifs ascribed to substrate recognition and catalysis are generally similar to those of vertebrate retroviral RTs, a notable difference is replacement of the highly-conserved active site His with Tyr in the RNase H domain of Ty3 RT. More significantly Ty3 RT lacks the connection, or tether, between its DNA polymerase and RNase H active sites, with the consequence that on the nucleic acid substrate they are separated by ~13 bp as opposed to the 17-18 bp observed for lentiviral and gammaretroviral enzymes. Structural similarity between the HIV-1 connection and its RNase H domain originally suggested the latter arose through domain duplication, while an alternative theory proposes the functional RNase H domain was acquired as a new, and more efficient element from a source outside the LTR retrotransposons. Structural and biochemical information on subdomain organization of Ty3 RT is therefore important in understanding the evolutionary divergence between these essential vertebrate retroviral and LTR-retrotransposon enzymes. To this end, we provide the first structure of Ty3 RT in complex with an RNA/DNA hybrid at 3.1 Å resolution. The fully-active enzyme is an asymmetric homodimer of 55 kDa subunits, and is formed only in the presence of the RNA/DNA substrate. Modeling the spatial separation between the DNA polymerase and RNase H active sites, coupled with site-directed mutagenesis, suggests that one active site of each subunit contributes enzyme activity.

BACK

F33. Mismatch Resolution of HIV-1 WT and AZT-Resistant Reverse Transcriptase Using a Forced Copy Choice Recombination System

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HIV-1 is a genetically diverse virus that can rapidly evolve, making prevention and treatment of infections difficult. A major source of the genetic variation found in HIV-1 populations is attributed to the error-prone process of reverse transcription. Unlike many DNA polymerases, retroviral reverse transcriptase (RT) lacks 3' to 5' proofreading exonuclease activity. While HIV-1 RT has the ability to extend incorporated mismatches, it can also excise terminally mismatched nucleotides. The excision capabilities of HIV-1 RT are responsible for resistance to the antiretroviral drug AZT. The nucleotide mismatch extension and excision reactions of RT have been studied *in vitro* but their role in viral replication *in vivo* remains unknown. Here, we developed a novel system to study the resolution of mismatched nucleotides by HIV-1 RT during viral replication in cultured cells. Retroviral inside-out (RIO) vectors were designed so that the initial minus strand reverse transcription product contained 30 nucleotides of complimentarity to a secondary acceptor template site on the RNA, thus forcing template switching at a defined location during viral reverse transcription. Initial characterization revealed that inside out vector RNAs were packaged efficiently, but that RIO vector proviral titers were 50-100 fold lower per packaged RNA than titers for canonical vectors. Single nucleotide substitutions were then introduced at acceptor template positions complementary to the 3' end of the transferring primer strand. Resolution of the resulting mismatched base pairs by wild-type (WT) or AZT-resistant (AZT^r) RT was examined after single rounds of replication using diagnostic restriction digests and sequencing of proviral products. The results demonstrated that both WT and AZT^r RTs were able to efficiently extend all of the mismatches tested except for the dC:rC mismatch. Interestingly, while WT RT preferentially resolved this mismatch by either excising the mismatched base or switching templates before the mismatched base at the 3' end was incorporated, the AZT^r RT more frequently processed the mismatch by realigning the DNA primer to an adjacent, matching nucleotide on the template. Another difference in mismatch processing between RTs occurred with the dT:rC mismatch. Both RTs extended the mismatch most of the time, but WT RT displayed 2- to 4-fold more base excision/premature jump products than the AZT^r RT. Overall, these results indicate that HIV-1 RT displays nucleotide-specific differences in mismatch extension during virus replication and that the mechanism of mismatch resolution differs between WT and AZT^r RTs.

BACK

F34. Structural Insights into RT Mutation Q151M that Confers Multi-Nucleoside Drug Resistance

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HIV-1 reverse transcriptase (RT) is targeted by multiple drugs. RT mutations emerge that confer resistance to the drugs in clinical use. Commonly observed RT mutations that emerge in response to nucleoside RT inhibitors (NRTIs) are K65R, M184V/I, excision enhancing mutations (EEMs, TAM or AZTr), insertions or deletions in the p66 fingers b3-b4 loop, and Q151M. The molecular mechanisms by which a mutation (or a set of mutations) confers resistance to NRTIs are distinct for each mutation.

The mutation Q151M, which occurs primarily in association with four other RT mutations (F116Y, F77L, V75I, A62V), is detected in patients failing therapies that include dideoxynucleosides (didanosine, ddI; zalcitabine, ddC) and/or zidovudine (AZT). The list of the five mutations (Q151M, F116Y, F77L, V75I, A62V) is referred to as the Q151M-complex (Q151Mc), and an RT or virus containing the Q151Mc mutations remains resistant to multiple NRTIs.

To understand the structural basis for Q151M (or Q151Mc) resistance, we systematically determined crystal structures of ternary complexes of (i) wild-type RT/dsDNA/dATP, (ii) wild-type RT/dsDNA/ddATP, (iii) Q151M RT/dsDNA/dATP, (iv) Q151Mc RT/dsDNA/dATP, and (v) Q151Mc RT/ddATP. The structures revealed that the deoxyribose rings of dATP and ddATP have two distinct conformations while the base and phosphate moieties of the RT-bound dATP and ddATP superimpose. The single mutation Q151M introduces conformational perturbation to the dNTP-binding pocket, and the pocket can have three distinct shapes due to three possible positions for the Cd atom of the flexible side-chain of M151. The compensatory set of mutations restricts the side-chain flexibility of M151 and restores the DNA polymerization efficiency of the enzyme. The detailed structural features and analysis of the molecular mechanisms of resistance Q151M and Q151Mc mutations will be presented.

BACK

F35. Rilpivirine Inhibition of HIV-1 RT Initiation: A Pre-Steady State Kinetic Approach

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Reverse transcription of the HIV-1 genomic RNA into DNA is an essential, complex and multi-step process of the viral life cycle. Initiation of reverse transcription starts by annealing 18 nucleotides from the 3'-end of cellular $tRNA^{Lys3}$ with a complementary sequence in the viral genome (primer binding site or PBS). The HIV-1 reverse transcriptase (RT) initiates DNA synthesis using $tRNA^{Lys3}$/viral RNA (vRNA) as primer-template (P/T). RT incorporates the first 5-6 nucleotides in a distributive manner before switching to processive elongation mode during initiation of reverse transcription. Due to the low efficiency of nucleotide incorporation during initiation, this step has been proposed to be an attractive antiviral target. While some kinetic characterization of NRTI inhibition of initiation has been carried out, there are no reports on the mechanism of NNRTI inhibition of this first step of reverse transcription. We used two independent transient kinetic methods (Rapid-Quench Flow and Stopped Flow) to determine the mechanism of rilpivirine (RPV) inhibition and resistance at the initiation of reverse transcription. The kinetic parameters of WT and E138K/M184I (clinical resistance mutations) in single nucleotide incorporation (initiation) and multiple nucleotide incorporation (elongation) reactions were determined using 25/76 $tRNA^{Lys3}$/vRNA and 18/25 RNA/RNA PBS-containing (P/T) substrates.

Our kinetic data showed that WT HIV-1 RT binds RNA/RNA and $tRNA^{Lys3}$/vRNA with ~5- and 10-fold less affinity, respectively compared to a DNA/DNA substrate of the same sequence. Resistance mutations E138K/M184I did not affect the binding affinity of $tRNA^{Lys3}$/vRNA or RNA/RNA P/T to HIV-1 RT. The polymerase efficiency of both WT and E138K/M184I enzymes at the initiation of reverse transcription were comparable to each other but remarkably lower ($k_{pol}/K_{d.dCTP}$ >100-fold) than on a DNA/DNA P/T substrate containing the same PBS sequence. The elongation kinetics data revealed that the HIV-1 integration complex undergoes conformational changes upon switching from the initiation to the elongation step of reverse transcription. In addition, the polymerase efficiency ($k_{pol}/K_{d.dCTP}$) of WT as well as the E138K/M184I mutant enzyme at the elongation step was significantly greater (~16-fold) compared to the initiation step.

The clinical resistance mutations (E138K/M184I) have decreased susceptibility to RPV due to decreased RPV binding affinity (~2.5-fold compared to WT HIV-1 RT) during initiation of reverse transcription. The decreased RPV binding affinity of E138K/M184I HIV-1 RT was mainly due to a faster dissociation rate (k_{off}) of RPV from the mutant enzyme (higher k_{off}). Interestingly, the association rates of RPV ($k_{on.RPV}$) to WT and mutant enzymes were comparable.

This work was supported in part by NIH/NIGMS P50 GM103368.

BACK

F36. Structural Studies on Understanding and Overcoming NRTIs' Drug Toxicity

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Tremendous success fighting human immunodeficiency virus replication and transmission with current antiviral drugs must now be balanced against the severe side effects of these reagents. Antiviral inhibitors of HIV reverse transcriptase (NRTIs) interfere with human mitochondrial DNA polymerase (Pol g) leading to cellular and tissue toxicity with respect to key cell biological pathological and pharmacological events. We determined crystal structures of human mitochondrial DNA polymerase halted by various antiviral inhibitors captured during DNA replication. Although NRTIs are structural similar, their toxicity to human varies by 6-order of magnitudes. Our structural results of Pol g-DNA-NRIT complexes enable us to correlate inhibitor structure with their drug toxicity. Comparison structures of NRITs bound to the target HIV-RT and adverse reaction target human Pol g provides insight on designs of low toxic and potent antiviral reagents.

F37. Structural Integrity of the Ribonuclease H Domain in HIV-1 Reverse Transcriptase

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Reverse Transcriptase (RT) is a viral enzyme which is essential to the replication process of Human Immunodeficiency Virus type 1 (HIV-1). The RT is encoded as a 66 kDa protein in the virus and is matured to form a heterodimer that is composed of 66 kDa (p66) and 51 kDa (p51) subunits. The p51 subunit is generated by proteolytic cleavage of the most of the ribonuclease H domain (RNH) from a p66 subunit by HIV-1 protease (PR). Since the p66 homodimer is known to be enzymatically active, it has been questioned why the heterodimer formation is necessary *in vivo*. Previous studies investigated the necessity of heterodimeric RT by introducing mutations at the RT p51-RNH PR processing site (p51↓) in an attempt to block proteolytic processing of p66 in the virus. Instead, significantly attenuated viruses that contain over-processed RT to p51 or the smaller fragments than the p51 were observed (1). Further study led to the identification of an additional compensatory mutation, T477A, which restores proteolytic stability of p66 in the RT p51↓ mutants (2). Here, we explore the structural basis for the observed differences in proteolytic stability in the RT p51↓ mutants and the T477A revertant mutants, primarily using solution NMR and computational methods. NMR results show that the RNHs with the p51↓ mutations are more unfolded compared to that of the wild type (WT). Addition of the T477A mutation restores the folding. Computation studies demonstrate the importance of processing site residues for the RNH structure. Overall, our results indicate that the amino acid sequence within the p51↓RNH cleavage site is critical for structural stability as well as substrate specificity in RT maturation.

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F38. Atomic Force Microscopy to Elucidate Sequence Context Dependent Interaction of APOBEC3G with ssDNA

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APOBEC3G (A3G) is capable of catalyzing multiple C to U lesions in nascent minus strand cDNA during HIV-1 reverse transcription. A3G is a double domain deaminase, with an N-terminal binding domain (NTD) and C-terminal catalytic domain (CTD). The enzyme has a nucleotide target site specificity for consecutive cytidines of a CCC motif, however the role of the sequence on the A3G binding to the target remains unclear. To answer this question we investigated the impact of nucleotide bases flanking the CCC motif in single stranded DNA (ssDNA) substrate on the A3G-DNA complex formation. Atomic force microscopy (AFM) along with the hybrid DNA design was employed. In this approach, DNA substrate contained a ssDNA tail, allowing us to discriminate specific and non-specific complexes as shown in Fig. 1. Two designs with ssDNA substrate containing two CCC motifs having either AAA (hot) or TTT (cold) nucleotide sequence in the immediate vicinity of the CCC motif were constructed. We have observed the complex formation for both designs, but the yields of complexes were slightly higher for the hot design. Additionally, the protein stoichiometry was different, but the effect is small in comparison to the 25-fold drop in the catalytic activities for the cold sequence compared to the hot one. Overall, the data suggests that sequence specificity can be involved in the complex formation, but it is not the major effect in the A3G-susbtrate interaction.

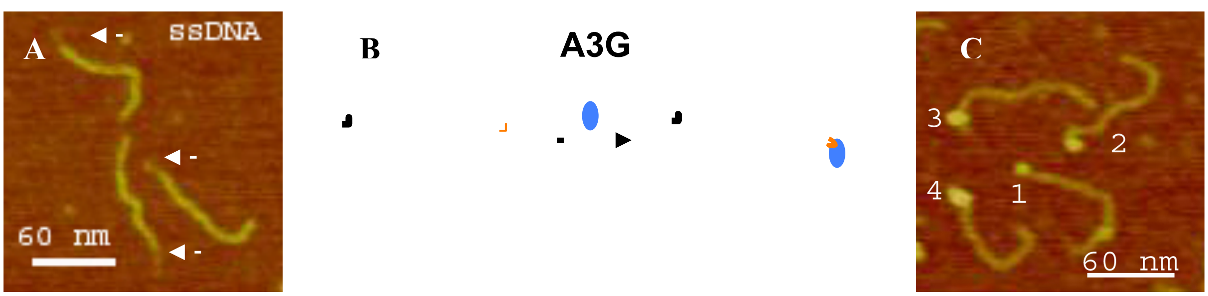

Figure 1: (A) AFM image of ssDNA construct of 69-nt attached to 441-bp double stranded DNA (dsDNA) acting as substrate for A3G. (B) Schematic for the experimental approach to image the A3G binding to ssDNA. (C) AFM image of complexes of A3G with ssDNA at the tail of dsDNA. Molecule labels: 1, monomer; 2, dimer; 3, trimer; 4; tetramer.

This work was supported by NIH P01 GM091743.

F39. Small Molecule Probes of APOBEC3-Catalyzed Mutation

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The APOBEC3 (A3) family of 7 DNA cytosine-to-uracil deaminases functions in innate immune defense by deaminating foreign and viral DNA. Inherent to the pro-mutagenic capacity of the A3 protective mechanism, low levels of A3-expression and catalysis, as dictated by virion infectively factor (Vif), have been implicated in driving the genetic diversity of HIV-1. Sub-lethal levels of A3-catalyzed mutation can enable viral escape from immune defense and/or the development of resistance to antiretroviral treatments.[1] Therefore, small molecule inhibitors of the A3 enzymes may offer a novel strategy in HIV-1 drug development by slowing the overall mutation rate of HIV-1 and thereby limiting the virus' ability to evolve. We predict that A3 inhibition will yield hypomutated HIV-1 with increased susceptibility to adaptive immune clearance and antiretroviral treatment.

An on-going endeavor by the Harki and Harris laboratories has accomplished the high-throughput screening (HTS) of over 500,000 small molecules for A3 inhibition. Thus far, our efforts have yielded four unique chemotypes as lead A3 inhibitors. Two scaffolds, as represented by A3G inhibitors MN30 and MN256, contain reactive functionalities that inhibit A3G by engaging C321, a residue proximal to the active site.[2,3] Two other scaffolds, as represented by MN132* and M136, inhibit multiple A3s through seemingly non-covalent mechanisms of action.[4,5] This poster will highlight our work to develop potent and selective A3 inhibitors through iterative analogue syntheses, biochemical evaluations, and mechanistic studies.

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BACK

F40. Small Molecule APOBEC3G Activators as a Novel Strategy for the Prevention of HIV Infection

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APOBEC3G (A3G) exerts its antiviral activity on HIV-1 by packaging in budding virions and mutating the viral genome during reverse transcription in subsequently infected cells. Previously, we showed that cellular RNAs inhibit the ability of A3G to both bind to and deaminate ssDNA substrate. As cellular A3G:RNA complexes have been shown to be inactive against incoming HIV, we hypothesized that decreasing cellular RNA binding by A3G will activate this enzyme to prevent the virus from establishing an infection. OyaGen has developed an *in vitro* quenched FRET (FqRET) assay for high-throughput screening (HTS) to identify small molecules that decrease A3G:RNA binding. With this screen, we developed lead compounds that function through the proposed mechanism of action of reducing RNA binding to A3G and activating cellular A3G ssDNA deaminase activity. A novel finding of this work is that treatment of cells with these compounds allows A3G to initiate a preemptive strike on incoming virus without its prior encapsidation. Cells tolerated these compounds above 100 mM, yet viral infectivity was markedly inhibited in cells pre-treated at only nanomolar concentrations. Thus, treatment with A3G activators may be an effective strategy for therapeutic intervention or as prophylaxis. Moreover, combinatorial treatment of an A3G activator with a Vif antagonist would represent a potent and novel therapeutic strategy to activate an innate host immunity that is refractory to drug resistance.

BACK

F41. The Dinucleotide Preference of APOBEC3G Is Dispensable for HIV-1 Restriction

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The DNA cytosine deaminases APOBEC3A (A3A) and APOBEC3G (A3G) are key players in the innate immune response. A3A restricts foreign DNA whereas A3G inhibits the replication of a variety of reverse transcribing pathogens including HIV-1. Both of these enzymes efficiently deaminate cytosines in single-stranded DNA but can be distinguished from each another by their local dinucleotide preferences. A3A prefers to deaminate cytosine in a TC dinucleotide context whereas A3G prefers a CC dinucleotide context. To assess the role of substrate preference in HIV-1 restriction, we changed A3G into a TC-preferring deaminase by replacing loop 7 in the catalytic domain with the analogous region from A3A. This chimeric enzyme showed potent HIV-1 restriction activity that was indistinguishable from that of the wildtype enzyme. These data support a model in which the loop 7 region governs the selection of local dinucleotide substrates for deamination but is unlikely to be part of the higher level targeting mechanisms that direct these enzymes to biological substrates such as HIV-1.

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F42. Inhibition of APOBEC3G Deaminase by Synthetic Peptides Sensitizes Lymphoma Cells to Genotoxic Agents

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The HIV-1 Vif protein neutralizes A3G's restriction activity to establish effective infection in the host target cells. Vif neutralizes A3G mostly by two mechanisms: As a primary mechanism, Vif hijacks a cellular protein complex that includes CBF-β, Cullin5 and ElonginB/ElonginC to degrade A3G via the ubiquitin-proteasome pathway. As a secondary mechanism, Vif directly controls the deamination threat in virions or in pre-integration complexes following entry to target cells. Previously we showed that purified HIV-1 Vif protein inhibits A3G activity *in vitro* at nanomolar concentrations in an uncompetitive manner. To identify the inhibitory domains in Vif, we screened a battery of 46 15-mer Vif-derived peptides with 11-aa overlapping and covering the full-length protein for the inhibition of A3G-mediated deamination. The peptides Vif25-39 and especially Vif107-119 significantly reduced the A3G activity at 1 μM and 0.2 μM with IC_{50} values of approximately 0.6 μM and 0.1 μM, respectively. To increase the efficiency of these inhibitory peptides, we truncated the peptides and found that a short peptide Vif107-115 restored the same inhibitory activity. This peptide bears a motif LYYF, which is present in A3F. Two A3F derived peptides consist with this motif inhibit the A3G activity. Interestingly the Vif107-115 and A3F305-311, which contain the motif LYYF reduce the binding of wild type and mutated A3G molecules to single-stranded DNA.

We have shown that A3G may also promote double strand break (DSB) repair by direct end synapsis and deamination-dependent cleavage of resected ssDNA. We show that treatment of ionizing-irradiation resistant lymphoma cells, which express A3G, with these inhibitory peptides, renders these lymphoma cells sensitive. These results suggest that A3G may be a potential therapeutic target amendable to peptide and peptidomimetic inhibition.

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BACK

F43. Cross-Species Analysis Reveals Differential Composition of the APOBEC3-Degrading Lentiviral Vif E3 Ligase Complex

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A fundamental goal of virological research is to determine how different viruses have overcome the myriad of challenges to replicate successfully in an infected host. Comparative studies between different viruses and hosts have been instrumental towards defining both key replication barriers as well as viral strategies for surmounting them. Here, we present a comparative analysis of complexes formed by representative Vif proteins from different lentiviral clades. We find that, while each Vif protein employs a similar strategy of recruiting an E3 ligase complex to degrade the antiviral APOBEC3 proteins, the composition of this complex varies by lentiviral clade. These results implicate potentially unique functional attributes gained by each Vif protein during their distinct evolutionary histories and demonstrate mechanistic flexibility in the strategies employed by different viruses to overcome the same host challenge.

Human Immunodeficiency Virus (HIV) Vif is known to overcome the antiviral APOBEC3 proteins by the recruitment of an E3 ubiquitin ligase complex that poly-ubiquitylates the APOBEC3 proteins, targeting them for proteasomal degradation. The HIV Vif E3 ligase complex is composed of not only the canonical Cullin5-RING ubiquitin ligase, but also requires additional recruitment of the transcription factor CBFβ. It is unclear why Vif has evolved to co-opt CBFβ, but it is hypothesized that this recruitment can alter the cellular transcriptional program to the benefit of the virus. While Vif proteins from the related lentiviruses all also target their host APOBEC3 proteins for degradation, it is unclear if they all do so by a similar means.

In this study, we performed affinity-purification mass spectrometry (AP-MS) to identify members of Vif-hijacked complexes across several lentiviral subclades. We found that while all lentiviral Vif species engage the Cullin5-RING ubiquitin ligase in human cells, only primate lentiviral Vif proteins recruit CBFβ. While some non-primate lentiviral Vif proteins appear to operate independently of any other factors, others require additional, novel complex members for both their *in vitro* reconstitution and *in vivo* APOBEC3-degrading activity. These data therefore demonstrate a mechanistic flexibility in viral rewiring of the host cell despite maintenance of a conserved activity.

BACK

F44. HIV-1 Vif Adaptation to Natural APOBEC3H Variants

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Humans encode seven APOBEC3 cytidine deaminases, of which some can restrict HIV-1. The single deaminase domain APOBEC3H (A3H) enzyme is highly polymorphic among human populations (> 7 haplotypes). Most A3H haplotype proteins are instable and have limited anti-retroviral activity, but A3H haplotype II (A3H-hapII) possesses strong activity against HIV-1.

We previously analyzed A3H variants and HIV adaptation in an HIV cohort consisting of 76 acutely HIV infected participants from the University of California. The comparison of plasma viremia patients with stable and unstable A3H variants showed a reduction in viral loads in the stable A3H group, indicating that stable A3H affects HIV *in vivo*. In addition, we found that patients expressing stable A3H variants more often harbored viral strains with Vif encoding a phenylalanine (F) at position 39 (60% had 39F in stable A3H hapII carriers versus 30% 39F in unstable A3H carriers). Remarkably, Vif 39F is critical for the efficient degradation of A3H. Moreover, we demonstrate that endogenously expressed A3H restricts HIV replication in primary CD4+ T-cells and that HIV restriction correlates with massive editing of proviral DNA.

To validate our previous findings in an independent cohort we analyzed Vif sequences and A3H haplotypes in the Amsterdam HIV cohort. Similar to the San Francisco cohort, 80% of the Amsterdam HIV cohort patients expressing stable A3H carried Vif 39F, compared to only 40% in unstable A3H carriers. It is conceivable that the activity of Vif to degrade A3H is determined by multiple residues. To test this possibility, we cloned patient-derived Vif variants in full length HIV and analyzed their infectivity in the presence of A3G and A3H. As expected, all Vifs efficiently counteract A3G. However, most Vif variants from inactive A3H carriers failed to counteract A3H, whereas the majority of active A3H carriers efficiently counteracted A3H. Vif sequence comparisons between Vifs differing in anti-A3H activity show that a phenylalanine at Vif position 39 is required to fully counteract A3H. In addition, we identify other critical Vif residues required to specifically counteract A3H. Modeling of these Vif residues on the Vif crystal structure shows they all cluster to one interface. Subsequent docking analyses show a Vif/A3H interface containing all the A3H-important Vif residues.

Combined, these data from two independent HIV cohorts show that HIV adapts to the genetically diverse restriction factor A3H in humans. We hypothesize that active A3H carriers infected with HIV unable to counteract A3H could control HIV replication.

F45. Species Specific Requirements for Lentivirus Vif Hijacked Ubiquitin Ligase Complex

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Among the known lentiviral accessory proteins, the virally encoded Vif protein is the most highly conserved and is found in all lentiviruses except equine infectious anemia virus (EIAV). The primary function of Vif is to hijack a cellular Cullin-RING ubiquitin ligase and target host APOBEC proteins for degradation by the 26S proteasome. Recent work has confirmed an essential interaction between primate lentiviral Vif and the cellular transcription factor CBF-β, and has shown that CBF- β is required for Vif mediated APOBEC degradation and viral infectivity. To determine which Vif-host interactions are conserved across lentiviruses, we employed an unbiased proteomics approach using affinity purification-mass spectrometry (AP-MS) to study five Vif proteins from Maedi-Visna Virus (MVV), Bovine Immunodeficiency Virus (BIV), Feline Immunodeficiency Virus (FIV), Simian Immunodeficiency Virus Macaque (SIVmac), and HIV-1. From these studies, we confirm that the CBF- β interaction is primate specific. Unlike HIV-1 Vif, the BIV Vif E3 ligase complex does not require any additional, non-Cullin-RING ubiquitin ligase host factors to efficiently ubiquitinate BIV APOBEC. However, both FIV and MVV appear to need a cellular co-factor to fold and function properly, and through our AP-MS approach we have identified a critical cellular factor that interacts with MVV Vif in the context of the MVV Vif E3 ligase. Here we are able to reconstitute the HIV, BIV, and MVV Vif ubiquitin ligase complexes and demonstrate enzymatic activity in an in vitro unbiquitination assays. Our results suggest that the functional BIV Vif E3 ligase may serve as a minimal model by interacting with only known Cullin-RING ubiquitin ligase components, whereas both HIV and MVV require additional cellular factors for efficient degradation of APOBEC. Together these studies further our understanding of Vif mediated APOBEC degradation and demonstrate important interactions between lentiviral Vifs and non-Cullin-RING ubiquitin ligase host factors.

F46. Towards Comprehensive Sequence-Function Maps for Vif

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We plan to determine the effects of all possible point mutations in Vif on the competitive fitness of HIV-1 amplification in cell culture. The results of these studies will provide a comprehensive map of Vif sequence-function relationships that will be valuable for identifying functional hot spots that are critical for Vif function and more broadly for understanding the biophysical properties required at each amino acid position in Vif. We have developed approaches to efficiently generate site-directed point mutant libraries in plasmid encoded HIV-1 and to quantify the competitive advantage/disadvantage of each mutation using bulk competitions and deep sequencing.

BACK

F47. Exploiting Viral-Host Variation to Define the HIV-1 Vif-A3G Interface

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HIV-1 Vif binds the host restriction factor APOBEC3G (A3G) and subsequently mediates its proteasomal degradation. Earlier studies suggested only a single loop, encompassing residues 128-130 in the N-terminal A3G domain, to interact with an expansive range of residues in the N-terminal region of Vif. The recently-published Vif crystal structure reveals these residues cover a large surface of Vif, suggesting the interaction between Vif and A3G extends beyond the A3G-128 loop.

Data from one study in 2006 indicated Vif residues 14-17 could specifically confer Vif activity against A3G-128 variants. To further study this point of contact, we mutated residues 14-17 in NL4-3 Vif and screened them against A3G 128 loop variants. Altering the charge of Vif residues 14-17 to counter the charge of A3G residue 128 allowed for Vif to counteract A3G in single cycle infectivity assays, confirming this specific interaction.

We next mapped the Vif requirements of HIV-1 Vif variants that differed in their ability to degrade the A3G-129Q, a protective A3G polymorphism found in gorillas. HIV-1 subtype C Vifs naturally counteracted A3G 129Q, whereas NL4-3 Vif was inactive. Infectivity and A3G degradation assays with HIV-1 subtype C and HIV-1 NL4-3 chimeras indicated Vif residue 23S specifically conferred activity against A3G-129Q but not A3G-128K.

To identify additional Vif binding sites in A3G we performed extensive arginine scanning of surface exposed N-terminal domain A3G residues, which suggest that the A3G residue 125, located in the domain for dimerization, RNA binding and viral incorporation, is critical for Vif mediated degradation. Screening this A3G mutant against a panel of patient-derived HIV-1 group B Vifs revealed that several Vif variants could counteract A3G-125R. Sequence analysis showed the A3G-125R active Vifs differed from the inactive Vifs at positions 19 and 22. Mutating these residues in the active Vifs rendered them inactive specifically against A3G-125R but not A3G-WT, indicating these residues are an additional point of contact between Vif and A3G.

We modeled the A3G N-terminal domain based on the A3C crystal structure and performed protein-docking analysis with the recently published Vif crystal structure. The predicted A3G/Vif interface includes all of the experimentally-derived points of contact and revealed that the A3G-128 loop binds a surface-exposed pocket in Vif.

BACK

F48. Viral Evolution and Directed Mutagenesis Studies Inform a Model for the Interaction of Human APOBEC3F with HIV-1 Vif

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Lentiviruses are a subset of retroviruses that produce illnesses characterized by a delay in the onset of symptoms following infection. Lentiviruses are responsible for causing a variety of diseases in mammals such as immunodeficiencies, blood cancers, and neuropathies. Mammals have evolved a variety of mechanisms to combat lentivirus infections. The APOBEC3 (A3) family of DNA cytosine deaminases function by directly attacking viral nucleic acid replication intermediates and converting normal DNA cytosine bases into mutagenic uracils. Every mammal has multiple A3 proteins that combine to suppress infection by lentiviruses. However, lentiviruses fight back by producing Vif which directly binds to CBFβ to recruit E3 ubiquitin ligase complex that mediates proteasomal degradation of A3 protein[1–3]. High-resolution structures of HIV-1 Vif[3] and several human A3 enzymes[4–8] are now available, but detailed information on the pathogen-host protein interaction surfaces have been elusive. Here, we use a combination of genetic, biochemical, and computational approaches to develop and test a model for the direct interaction between Vif and A3F. First, Vif-resistant A3F-E324K was used to select compensatory adaptations in Vif in long-term infection experiments. A single amino acid change restored Vif function and served as an anchor for docking studies. A combination of published and new site directed mutation data were used to distinguish between multiple interaction models and deduce the most likely binding interface. These surfaces are attractive targets for drug development with the long-term goal of identifying small molecules that disrupt the interaction with Vif and suppress HIV-1 infection through A3-mediated restriction.

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BACK

F49. Novel HIV Drug Screening Targeting the Interaction Between HIV-1 Vif and CBFβ

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OyaGen has developed a novel, FRET-based, live cell primary assay for high-throughput screening (HTS) to identify small molecules that bind to and inhibit protein domains that are critical for Vif binding to CBFβ. Our goal is to identify one or more high affinity molecular compounds that interact with either CBFβ or Vif in order to prevent CBFβ stabilization of Vif thereby disabling Vif's crucial role in HIV infectivity. Secondary assays and counter screens will verify that primary screen hits are specific inhibitors of Vif binding to CBFβ and have the anticipated effects on Vif destabilization and A3G protein stability as well as reduced viral infectivity. Counter screens will ensure that the compounds are not affecting the CBFβ interaction with RUNX1 and transcription. Furthermore, molecular modeling of lead compounds into the recently solved crystal structure of Vif in complex with CBFb will be utilized to assess probable binding surfaces of the compound in order to guide medicinal chemistry to improve binding affinity and efficacy. The goal is to identify non-toxic Vif-targeting compounds as leads for pre-clinical development of novel anti-HIV therapeutics.

F50. In Silico Molecular Screening of the HIV-1 Vif Protein to Enable Drug Discovery

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HIV-1 Viral infectivity factor (Vif) is a viral accessory protein that is absolutely required for HIV-1 infection in CD4 positive T cells, macrophages and dendritic cells due largely to its role in recruiting several antiretroviral factors of the APOBEC3 family to an E3 ubiquitin ligase complex for polyubiquitination and proteasomal degradation. Structure-assisted drug design is guided by the novel fold revealed by the recent publication of the crystal structure of Vif in complex with components of the E3 ubiquitin ligase complex. *In silico* molecular docking of small molecules with the 3D structures of target proteins serves as a complement to classic *in vitro* small molecule screening and has become a standard method to rank the predicted bound conformations and binding affinities of drug-like compounds in large virtual library screens. Here we report on *in silico* screening efforts to identify small molecules that bind to the surface of Vif and are predicted to interrupt specific protein-protein interactions that are essential for Vif function and HIV-1 infectivity.

F51. Fab Assisted Electron Microscopy of HIV Vif Complexes

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Advances in single particle cryo-EM have allowed atomic level resolution structures to be obtained of large assemblies. We recently described an approach that allows the technique to be applied to smaller particles (~100 kDa) by screening for fragment antigen binding (Fab) that form a stable, rigid complex with a target protein (Wu et al, Structure 2012). We describe application of this technology to HIV Vif-host complexes. We identified several promising Fab fragments and have characterized them by biochemical studies and negative stain EM. 2D reconstructions indicate this approach may be promising for resolution of complexes between A3 and the Vif E3 ubiquitin ligase.

BACK

F52. Ubiquitin Regulation of ZAP-70 Activity and Modulation by HIV Infection

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The tyrosine kinase ZAP-70 is a critical part of the pathway to initiate T-cell response. Regulation of ZAP-70 by post-translational modifications is important for normal T-cell response and could be a target of HIV infection. Interestingly, one important regulatory ZAP-70 modification appears to be ubiquitination. Preliminary data shows that there are many ZAP-70 ubiquitination sites that do not seem to lead to the degradation typically associated with ubiquitination. Some of these sites are also differentially ubiquitinated in HIV infected cells and in the presence of the HIV Viral infectivity factor (Vif) and therefore may be an important mechanism by which HIV interferes with normal T-cell response.

Non degradatory ubiquitination is not well understood, however several of the ZAP-70 ubiquitination sites have implications for ZAP-70 activation based on their location on the ZAP-70 structure and proximity to known mutations. We use molecular dynamics simulations to explore the mechanism by which ZAP-70 ubiquitination can allosterically propagate through the protein and affect activity. Methods from information theory help us to identify conformational coupling between non-adjacent sites on a protein. This may allow us to predict the effect of known ZAP-70 post-translational modifications and identify sites that may have an allosteric effect when modified or mutated.

BACK

F53. Novel Structural Regulation for Substrates-Controlled GTP-Activated SAMHD1 Deoxynucleoside Triphosphate Triphosphohydrolase

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SAMHD1 is the only known eukaryotic deoxynucleoside triphosphate triphosphohydrolase (dNTPase) and a major regulator of intracellular dNTP pools. Maintenance of the optimal intracellular concentrations of deoxyribonucleotides (dNTPs) is critical for the survival of eukaryotes, and SAMHD1 creates a low dNTP concentration status in non-cycling cells to inhibit viral DNA synthesis of diverse viruses. How SAMHD1 dNTPase activity is regulated is not yet clear. Here we report high resolution structures of SAMHD1 GTP-bound dimer and GTP/dATP bound SAMHD1 tetramer. Our data show that human SAMHD1 contains two unique activator binding sites in the allosteric pocket of the active tetramer. The primary activator GTP binds to one site, and substrate dNTP occupies the other. Consequently, both GTP and dNTP are required for activation of the enzyme. In the absence of substrate binding, SAMHD1 adopts an inactive conformation even when complexed with GTP. Furthermore, SAMHD1 activation is regulated by the concentration of dNTP. Guided by structural information, we have further demonstrated that substrate binding in the allosteric pocket is essential for hydrolase activity. Thus, the level of dNTP pools is elegantly regulated by SAMHD1's self-sensing ability through a novel activation mechanism.

BACK

F54. Binding of HIV-1 Vpr to the Human Homolog of the Yeast DNA Repair Protein RAD23 (hHR23A) Requires its XPC Binding (XPCB) as well as the Ubiquitin Associated 2 (UBA2) Domains

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The human homolog of the yeast DNA repair protein RAD23, hHR23A, was previously found to interact with the HIV-1 accessory protein Vpr. hHR23A is a modular protein containing an N-terminal ubiquitin-like (UBL) domain and two ubiquitin associated domains (UBA1 and UBA2), separated by a xeroderma pigmentosum complementation group C binding (XPCB) domain. All domains are connected by flexible linkers. hHR23A binds ubiquitinated proteins and acts as a shuttling factor to the proteasome. Here, we show that hHR23A utilizes both the UBA2 and XPCB domains to form a stable complex with Vpr, linking Vpr directly to cellular DNA repair pathways, and their probable exploitation by the virus. Detailed structural mapping of the Vpr contacts on hHR23A, by NMR, revealed substantial contact surfaces on the UBA2 and XPCB domains. In addition, Vpr binding disrupts an intra-molecular UBL-UBA2 interaction. We also show that Lys48-linked di-ubiquitin (di-Ub^{K48}), when binding to UBA1, does not release the bound Vpr from the hHR23A/Vpr complex. Instead, a ternary hHR23A/Vpr/di-Ub^{K48} complex is formed, indicating that Vpr does not necessarily abolish hHR23A-mediated shuttling to the proteasome.

BACK

F55. Elucidating the Evolutionary History of Lentiviral Vpr And Vpx Interactions with Host Proteins

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The lentiviral accessory proteins Vpr and Vpx enhance the viral lifecycle through recruitment of host protein complexes. Vpr is found in all extant primate lentiviruses, while Vpx is found in only two lineages, including HIV-2. To understand how these viral-host interactions shaped Vpr/Vpx evolution and function, we have investigated the conservation of these protein-protein interactions throughout primate lentiviral evolution.

Using a phylogenetic framework of Vpr and Vpx proteins from diverse SIVs, we analyzed the ability of Vpr and Vpx orthologs to interact with three known HIV-1 and HIV-2 Vpr/Vpx interacting partners: the Cul4-DCAF1 ubiquitin ligase complex, the restriction factor SAMHD1, and the SLX4 complex of structure-specific nucleases. We discovered that all Vpr and Vpx orthologs tested are able to interact with the human Cul4-DCAF1 complex. This indicates that interaction with the Cul4-DCAF1 complex is a conserved ancestral function of Vpr/Vpx. We found that all Vpx, and a subset of Vpr proteins, interact with and degrade SAMHD1 from their autologous host. Furthermore, an additional subset of Vpr proteins can interact with the SLX4 complex. Consistent with these interactions, we have identified signatures of rapid host protein evolution on both SAMHD1 and a subset of SLX4 complex proteins, indicative of viral-host conflict. From this data, we have developed a model for the stepwise evolution of Vpr and Vpx functions, as well as the impact they have had on host proteins. Together, our data helps to understand the complex evolution of these important viral accessory proteins, along with the concomitant evolution of their target host proteins.

BACK

F56. GPS-Prot: A Visual Explorer for HIV and Human Protein Interactions

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Many critical cellular processes are carried out by the assembly of proteins into multi-protein complexes that range from binary interactions to much larger assemblies. Pathogens such as HIV, with limited genomes, greatly expand their functional repertoire by interacting with host proteins and their complexes. Proteomic and genomic studies in recent years have provided many candidate HIV-host complexes for further examination, including 435 host proteins identified from a broad mass spectrometry survey in the HARC Center. The number of human protein-protein interactions (PPIs) in general has grown tremendously and now stands at ~175,000 unique interactions distributed across a number of databases. We developed a visual protein interaction explorer, GPS-Prot (www.gpsprot.org), which automates the task of visualizing experimental HIV-host and human interaction networks and integrating them with other information, as well as making supporting publications readily accessible. One need only input a query gene identifier to generate a comprehensive network of experimentally-determined binding partners (e.g. HARC Proteomics, DIP, BioGRID, IntAct, MINT and more). Genome-wide RNAi screen hits can be overlaid on networks automatically. Confidence-scoring of PPIs (HIPPIE database) allows filtering of the most well-determined complexes from dense networks, and in some cases, can substitute for curated human complex information. HIV-host scoring is currently being refined. GPS-Prot also provides a convenient interface for users to upload and view their own data. GPS-Prot has applications as a general web-based PPI viewer and can be included as a pop-up or plugin on any site, as demonstrated by integration into the Bio-GPS gene annotation portal.

BACK

F57. HINT: Uncovering the Early Immune Response to HIV-1 Infection

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The HIV Immune Networks Team (HINT) was formed with the goal of uncovering early viral evasion strategies and emergent properties of the innate immune response to HIV-1 infection. We hypothesize that the balance between host response and viral counter measures is determinant of viral transmission. To understand the molecular mechanisms that underlie early infection events, we have employed integrated systems-based and computational modeling approaches, enabling the elucidation of cellular rewiring and responses following viral challenge. For example, assimilation of RNAi screening datasets with networks generated through global interactomics and post-translational modification analyses have revealed emergent properties that underlie virus and host signaling pathways and implicate potential therapeutic targets. These host-pathogen circuits are further employed to evaluate their impact upon the kinetics of replication through computational modeling strategies, as well as analysis of rare variants through exome sequencing to identify polymorphisms that may impact viral transmission. Overall, the collaborative efforts of our groups have resulted in global mechanistic insights towards innate immune response to HIV-1 and host-pathogen interactions may underlie early transmission events. We expect that these studies provide novel therapeutic targets for the development of vaccine, adjuvants, and prophylactic antivirals.

F58. PQBP-1 is an Innate Receptor for HIV-1

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Innate immune responses that trigger type-I interferon (IFN) secretion have been implicated in HIV-1 transmission and pathogenesis. HIV-1 has evolved countermeasures to escape the activities of several IFN-stimulated genes (ISGs), and those mechanisms not disabled by the virus define both cell type and species tropisms. Recent data suggest that one or more intrinsic signaling pathways sense invariant features encoded by HIV-1 and initiate innate immune responses, including IFN secretion. This response is proximally mediated by recognition of specific viral components in infected cells by pattern recognition receptors (PRR), resulting in the activation of transcription factors that participate in ISG expression and IFN synthesis, such as IRF3. Dendritic cells (DCs) play a critical role in the immune response to viral infection through the facilitation of cell intrinsic antiviral activity and the activation of adaptive immunity. Productive infection of DCs by HIV-1 triggers an IRF3-dependent innate immune response, which has recently been shown to require the activity of cyclic GAMP synthase (cGAS). Here, we report the results of a targeted RNAi screen utilizing primary human monocyte-derived DCs (MDDCs) to identify cellular immune regulators that directly interface with HIV-1-encoded features to initiate this innate response. One of the strongest candidates that emerged from this screen was polyglutamine binding protein 1 (PQBP1). We found that PQBP1 is required for IRF3-dependent signaling in HIV-1-infected MDDCs and human cell lines. PQBP1 directly binds to reverse-transcribed HIV-1 DNA and interacts with cGAS to initiate an IRF3-dependent innate response in myeloid cells. Our results demonstrate that PQBP1 acts as a proximal sensor of a pathogen-associated molecular pattern (PAMP) encoded by HIV-1 DNA to activate cGAS/IRF3-dependent antiviral responses.

BACK

F59. Identification of Host Cell Mechanisms Restricting HIV-1 Replication

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Replication of HIV is heavily interconnected with components of the host cell machinery. These may encompass inducible restriction factors, governed by innate immune circuitries, that act to eliminate pathogen burden. Further, constitutively active cellular components also appear to limit maximal levels of pathogen replication. We hypothesize that the latter enables the host to tolerate infection by attenuating adverse effects of unrestricted replication. The activities of both sets of factors likely determine the ability of a virus to establish a pathogenic infection. However, the compendium of factors involved in the control of HIV replication is not known.

To identify constitutively expressed factors that limit HIV replication we utilized an integrative bioinformatic approach, selecting a set of cellular genes that have the potential to impede viral replication. These genes were tested in siRNA-based loss-of-function assays, leading to the identification of 139 factors that interfere with HIV replication. A subset of these factors were subjected to additional validation and mapped to specific stages of the viral life cycle. So far, 14 factors were found to restrict HIV replication at the stage of viral transcription. One of these genes, BIRC2, limits HIV replication through an NF-κB-dependent mechanism. Since BIRC2 is a negative regulator of the non-canonical NF-κB pathway this indicates a positive role of non-canonical NF-κB signaling in HIV transcription. Additional experiments showed that stimulation of non-canonical NF-κB signaling enhances HIV replication. Interestingly, treating latently infected cells with a BIRC2 antagonist triggered reactivation of the virus in a latency model system.

While previous studies have demonstrated that canonical NF-κB signaling is utilized by HIV to increase viral replication, little research has been performed to determine whether the non-canonical NF-κB pathway is permissive or restrictive to HIV infection. Taken together, our findings strongly indicate that non-canonical NF-κB signaling is beneficial to HIV replication. Moreover, targeting BIRC2 or other components of this pathway may represent a novel approach for the reactivation of latent HIV-1.

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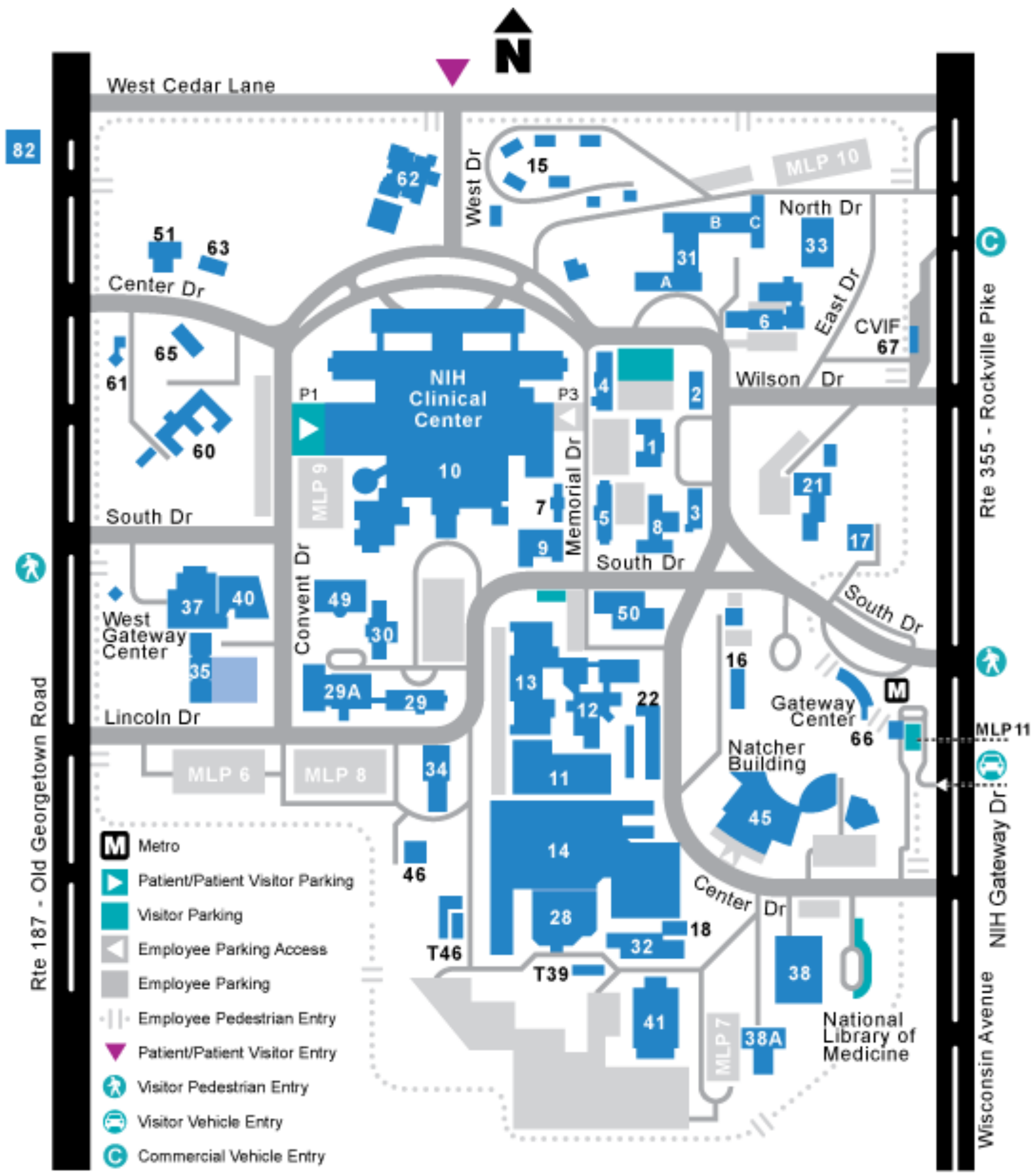
Map of NIH Campus



Some useful notes:

- The conference is located in Natcher (Building 45), southwest of the Gateway Center entrance to campus. The Medical Center Metro stop is next to the Gateway Center.
- Non-NIH employees must undergo inspection and receive temporary ID cards at the Gateway Center (see additional information on following pages).
- Parking garage MLP-11 is for non-NIH employees; NIH employees may park in the garage under Building 45 (requires car safety inspection next to Building 38A) or in other employee parking.
- Dining options on the NIH campus include Eurest Dining Services locations in Buildings 45, 1, 10, and 31, Maryland Business Enterprise Program for the Blind locations in Buildings 38A and 12B, and a concession stand in the Natcher lobby (http://does.ors.od.nih.gov/food/index.htm).
- An ATM is located in the Natcher lobby.

NIH Gateway Center Map

Main Visitor Entrance: NIH Gateway Drive
Gateway Center - Building 66 (for pedestrians entering campus)

Gateway Inspection Station - Building 66A (for vehicles entering campus)

• Monday – Friday: 5am – 10pm; Weekends and After Hours: Closed After hours: After 10pm on weekdays, all day weekends and holidays, pedestrians and visitors in vehicles should enter campus via the Commercial Vehicle Inspection Facility (CVIF) - Building 67 (on Rockville Pike between North Drive and Wilson Drive)
• After inspection, vehicles enter campus at Center Drive
• Roadway at Center Drive is for entering campus only; visitors exiting campus may exit from other open locations. To see a list of exits, please see the map.
• All vehicles and their contents will be inspected upon entering the campus.

Multi-Level Parking Garage 11 – MLP-11 (for parking outside of campus)

- Monday – Friday: 6am – 9pm (entrance) 6am – 11pm (exit) Weekends: Closed
- When MLP-11 is closed, visitors can park in lots on the NIH Campus
- Visitors parking in this garage should proceed to the Gateway Center (Bldg. 66) to get a visitor badge
- All visitors traveling in a vehicle are highly encouraged to park in MLP-11 as there is limited visitor parking on the main campus
- No vehicle inspection required to park in MLP-11
- Vehicles left in the MLP-11 parking garage after 11pm on weekdays or during any weekends are subject to ticketing and towing
- Cost: $2 per hour for the first three hours, $12 maximum for the entire day

Directions to NIH Gateway Drive from Rockville Pike/Wisconsin Avenue:
Southbound:

1. Continue on Rockville Pike past South Drive
2. Turn right at NIH Gateway Drive

Northbound – Option 1:

1. Continue on Rockville Pike past South Drive
2. Make a u-turn from the left turn lane at Wilson Drive
3. Continue southbound on Rockville Pike past South Drive
4. Turn right at NIH Gateway Drive

Northbound – Option 2:

1. Continue on Rockville Pike
2. Turn left at Battery Lane
3. Turn right on Old Georgetown Road
4. Turn right on Cedar Lane
5. Turn right on Rockville Pike
6. Continue southbound on Rockville Pike past South Drive
7. Turn right at NIH Gateway Drive

Northbound – Option 3:

1. Continue on Rockville Pike to South Drive
2. Make a u-turn from the left turn lane at South Drive
3. Continue southbound on Rockville Pike
4. Turn right at NIH Gateway Drive

Security Procedures for Entering the NIH Campus:
* All visitors and patients—**please be aware**: Federal law prohibits the following items on Federal property: firearms, explosives, archery equipment, dangerous weapons, knives with blades over 2 ½ inches, alcoholic beverages and open containers of alcohol.
* The NIH has implemented security measures to help ensure the safety of our patients, employees, guests and facilities. All visitors must enter through the NIH Gateway Center at Metro or the West Gateway Center. You will be asked to submit to a vehicle or personal inspection.
* Visitors over 15 years of age must provide a form of government-issued ID such as a driver's license or passport. Visitors under 16 years of age must be accompanied by an adult.

Vehicle Inspections – All vehicles and their contents will be inspected upon entering the campus. Additionally, all vehicles entering certain parking areas will be inspected, regardless of any prior inspection. Drivers will be required to present their driver's license and may be asked to open the trunk and hood. If you are physically unable to perform this function, please inform the inspector and they will assist you.
Vehicle inspection may consist of any combination of the following: Detection Dogs Teams (K-9), Electronic Detection Devices and Manual Inspection.
After inspection, you will be issued a vehicle inspection pass. It must be displayed on your vehicle's dashboard while you are on campus. The inspection pass is not a "parking permit." It only grants your vehicle access to enter the campus. You can only park in designated parking areas.

Personal Inspections – All visitors should be prepared to submit to a personal inspection prior to entering the campus. These inspections may be conducted with a handheld monitoring device, a metal detector and by visible inspection. Additionally, your personal belongings may be inspected and passed through an x-ray machine.

Visitor passes must be prominently displayed at all times while on the NIH campus.
To learn more about visitor and security issues at the NIH, visit:
http://www.nih.gov/about/visitor/index.htm.
For questions about campus access, please contact the ORS Information Line at orsinfo@mail.nih.gov or 301-594-6677, TTY - 301-435-1908.